



Cryptic or underworked? Taxonomic revision of the *Antistrophus rufus* species complex (Cynipoidea, Aulacideini)

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Abstract

Cryptic species present challenges across many subdisciplines of biology. Not all “cryptic” species, however, are truly cryptic; many are simply underexplored morphologically. We examined this idea for the *Antistrophus rufus* species complex, which previously contained three species thought to be morphologically cryptic. To determine whether the *A. rufus* complex are truly cryptic species, we assessed species boundaries of members of the *A. rufus* species complex using morphological, ecological, and DNA barcode data, and tested whether a set of 50 morphological characters could adequately diagnose these species. We revealed that this complex includes five species, and that there are useful phenotypic diagnostic characters for all members of this species complex. This enabled redescription of four species and the description of *Antistrophus laurenae* Nastasi, sp. nov., which induces externally inconspicuous galls in stems of *Silphium integrifolium* Michx., a host not associated with other members of the complex. We use these new diagnostic characters to construct a key to the five species of the *rufus* complex. We conclude that the *A. rufus* complex was not a true case of cryptic species. Our Bayesian analysis of DNA barcode data suggests possible cospeciation of members of the *rufus* complex and their *Silphium* host plants, but further study is necessary to better understand the evolution of host use in the lineage.

Keywords

Cryptic species, gall wasp, morphology, *Silphium*, superficial description impediment

Introduction

Cryptic species are those that cannot readily be distinguished on the basis of phenotypic variation alone (Struck et al. 2018); they have created challenges in myriad areas of the life sciences, especially in agro-economic systems (Andrews et al. 2020; Hansen et al. 2021; MacLeod et al. 2021). One such system of emerging significance is that of the genus *Silphium* L., a genus of composite herbs in the sunflower tribe (Asteraceae: Heliantheae) that is currently being investigated for use as biofuels, oilseeds, and additional economic purposes (Kowalski et al. 2013; Jasinskas et al. 2014; Peni et al. 2020; Vilela et al. 2020; von Cossel et al. 2020; Tîtei et al. 2021). Despite recent interest in cultivating and domesticating various *Silphium* species, as well as their prominence in threatened tallgrass prairie habitats, their associated arthropod fauna remains poorly studied (Henderson and Sauer 2010; Buffington et al. 2017). A major component of *Silphium* communities is herb gall wasps of the genus *Antistrophus* Walsh, 1869 (Fig. 1), which includes a known case of cryptic species (Tooker et al. 2004).

Six described species of *Antistrophus* induce galls in the disc flowers and stems of four *Silphium* species (Nastasi and Deans 2021). Perhaps the most intriguing *Silphium* gall wasps are three cryptic species composing the *rufus* species complex (*A. jeanae* Tooker and Hanks, *A. meganae* Tooker and Hanks, and *A. rufus* Gillette). These species are not only thought to be morphologically cryptic, but also induce galls that are inconspicuous and do not externally deform the host plant's tissues (often referred to as "cryptic galls" in the literature; e.g., Ronquist and Liljeblad 2001); each species induces these galls within stems of their respective host species of the genus *Silphium* (Tooker et al. 2004; Nastasi and Deans 2021). The complex was described by Tooker et al. (2004), who found each species to be distinct based on allozyme and ecological data, including use of host plant species (Tooker et al. 2004); *A. jeanae* is associated with *S. perfoliatum* L., *A. meganae* is associated with *S. terebinthinaceum* Jacq., and *A. rufus* is associated with *S. laciniatum* L. These species would be considered cryptic, or functionally so, based on the diagnostic morphological criteria which included averages of precise ratios of antennomere dimensions, lengths of dissected ovipositors, depths of galls within host stems, and masses of mature larvae (Tooker et al. 2004). These metrics are hard to replicate and do not fully diagnose the species, thus the original diagnoses are arguably insufficient to reliably arrive at a species identification without host plant data. While these are the only morphological diagnostic characters given by Tooker et al. (2004), the descriptions of these species, including Gillette's 1891 description of *A. rufus*, are fairly limited in the number of morphological characters examined. Due to the brief original descriptions of species composing the *A. rufus* complex, it seems



Figure 1. Examples of *Silphium*-galling *Antistrophus* wasps and their galls **A** adult female *A. jeanae* Tooker & Hanks, 2004 **B** galls and larvae of *A. jeanae* in stem pith of *S. perfoliatum* L. **C** adult female *A. silphii* Gillette, 1891 **D** galls of *A. silphii* on terminal stem of *S. integrifolium* Michx **E** adult female *A. laciniatus* Gillette, 1891 **F** galls of *A. laciniatus* in disc flower of *S. laciniatum* L. adult gall wasps photographed by Antoine Guiguet. Galls photographed by Andrew R. Deans.

likely that this is a case of the so-called “superficial description impediment”, a taxonomic phenomenon in which species are not readily identifiable based on characterization in existing literature (Meier et al. 2021), rather than a true case of cryptic species.

The potential for a superficial description impediment in the *A. rufus* complex is supported by the minimal history of taxonomic investigation of herb gall wasps in North America; among the early diverging lineages of gall wasps are several tribes of non-oak herbaceous gallers (Aylacini sensu lato), but these remain more poorly understood than the species-rich oak gallers (Cynipini). While the herb gall wasp fauna of the Western Palearctic has been comparatively well studied (e.g., Nieves-Aldrey 1994; Nieves-Aldrey et al. 2008; Nieves-Aldrey 2012; Nieves-Aldrey 2022), those in North America remain poorly investigated. Eighteen valid native species are known (Nastasi and Deans 2021; Nastasi et al. 2024); only 14 published papers explore genus- or species-level taxonomy of these taxa, and many of these papers treat only a single species (Table 1). Of these works, only two (Nieves-Aldrey 1994; Tooker et al. 2004) have been published in the last 100 years. When combined with the known diagnostic characters for the *rufus* complex, the paucity of taxonomic work on this group further increases the likelihood of a taxonomic impediment rather than a true case of the cryptic species phenomenon.

In our analysis of the *Antistrophus rufus* complex, we included *A. jeanae* (gall inducer on *S. perfoliatum*), *A. meganae* (gall inducer on *S. terebinthinaceum*), and *A. rufus* (gall inducer on *S. laciniatum*) by sampling individuals reared from stems of these three *Silphium* host species. In the process we reveal in the stems of *S. integrifolium* the presence of an additional species that we newly describe, *Antistrophus laurenae* Nastasi sp. nov.; *S. integrifolium* is a new host plant for the *rufus* complex. We include in this analysis *A. minor* Gillette, 1891; we treat this species in the *rufus* complex based on its adherence to the diagnostic criteria for the *rufus* complex

Table 1. Taxonomic treatments of North American herb gall wasps, 1869–present. Names later transferred to other genera marked with (*). Names later synonymized marked with (◊). Species that are non-native to North America (or questionably so) are excluded.

Author and year	Description of taxonomic work
Riley and Walsh 1869	Described <i>Antistrophus pisum</i>
Ashmead 1887	Described <i>Aylax harringtoni</i> *
Bassett 1890	Described <i>Aylax tumidus</i> * and <i>Aylax podagrae</i> *
Gillette 1891	Described <i>Aylax bicolor</i> *◊ and five species of <i>Antistrophus</i>
Brodie 1892	Described <i>Aylax nabali</i> *
Ashmead 1896	Described <i>Aylax ambrosiaecola</i> *, <i>A. cavicola</i> *◊, <i>A. mulgediicola</i> *◊, and <i>A. sonchicola</i> *◊
Ashmead 1897	Described the genus <i>Aulacidea</i> for some species placed in <i>Aylax</i>
Beutenmüller 1908	Described <i>Aylax chrysothamni</i> *
Beutenmüller 1910a	Reviewed North American species of <i>Aylax</i> sensu lato
Beutenmüller 1910b	Reviewed North American species of <i>Aulacidea</i>
Kinsey 1920	Described <i>Aulacidea abdita</i> and <i>A. annulata</i>
McCracken and Egbert 1922	Described <i>Aylax microseris</i> *
Nieves-Aldrey 1994	Revised genera of “Aylacini” including those in North America
Tooker et al. 2004	Described <i>Antistrophus rufus</i> species complex including <i>A. jeanae</i> and <i>A. meganae</i>

we propose here along with that of Tooker et al. (2004). *Antistrophus minor* induces inconspicuous, externally imperceptible galls like those of *A. rufus* in the same host plant species (*S. laciniatum*). While some authors have discussed these species as potential synonyms (e.g., Beutenmüller 1910a), no nomenclatural actions have been published. *Antistrophus minor* was excluded from Tooker et al.'s (2004) description of the *A. rufus* complex, but because its status as a valid species remained unclear, we opted to treat *A. minor* here.

Methods

Gall collection and rearing

In late autumn and winter of 2020, 2021, and 2022, we collected entire, senesced stems of *Silphium integrifolium*, *S. laciniatum*, *S. perfoliatum*, and *S. terebinthinaceum* from sites in Illinois, Indiana, Iowa, and Ohio (details below). We identified plants in the field based on external morphology, as the *Silphium* species we studied are easily diagnosable in our sampling region by characters of the leaves and stems that are observable even in senescent plants (e.g., Brock and Weakley 2020). At each site, we confirmed via stem dissection that externally inconspicuous stem galls were present in each host plant species. We then collected additional stems, cut them into evenly sized pieces, labeled them, and stored them in plastic zip bags, which we aerated by poking holes in them using insect pins. We returned stems to lab facilities at The Pennsylvania State University (University Park, Pennsylvania, USA), where we stored bagged stem samples in a barn (without climate controls) to expose occupants to natural environmental conditions. From April through September, we checked stems every two to three days for emerging insects, which we collected into vials containing ethanol and stored at -20 °C. For morphological study, we air-dried and mounted selected individuals. Nastasi (2023) provides further recommendations for rearing cynipid galls on herbaceous plants.

Museum collection material

We examined specimens from the following collections:

- AMNH** American Museum of Natural History, New York, NY, USA
INHS Illinois Natural History Survey, University of Illinois, Champaign, IL, USA
PSUC Frost Entomological Museum, The Pennsylvania State University, University Park, PA, USA
USNM National Museum of Natural History, Washington, DC, USA
WIRC Wisconsin Insect Research Collection, University of Wisconsin, Madison, WI, USA

Using Darwin Core biodiversity data standards (Wieczorek et al. 2012), we digitized label data of all specimens that we examined. Digitized specimen data for all individuals we examined, including plant sample numbers and exact emergence dates for newly reared material, are available in Suppl. material 1: table 1.

Morphological character selection, description, and examination

To assess morphological boundaries of species belonging to the *rufus* complex, we selected 50 morphological characters (Suppl. material 1: table 2) from contemporary works on taxonomy of herb gall wasps (i.e., Nieves-Aldrey 1994; Tooker et al. 2004; Melika 2006; Ronquist et al. 2015; Azmaz and Katılmış 2020; Buffington et al. 2020; Nieves-Aldrey 2022; Tavakoli et al. 2022). We selected characters based on perceived potential for species-level diagnosis as well as ubiquity across the taxonomic treatments that we considered. We matched anatomical terms for adult wasps to concepts in the Hymenoptera Anatomy Ontology (Yoder et al. 2010) and provide a URI table (see Seltmann et al. 2012) outlining morphological terminology (Suppl. material 1: table 3). Our terminology relating to cuticular surface sculpture follows Harris (1979). We used the following additional abbreviation that is absent from the Hymenoptera Anatomy Ontology:

- **DLO** (diameter of lateral ocellus) for the largest possible diameter of either lateral ocellus.

We evaluated each character for five females and five males of each species (“primary morphological exemplars;” Suppl. material 1: table 1); we putatively identified these individuals based on the associated host plant species and characters presented in the original descriptions and scored the primary type and some secondary types for each species. We selected specimens based on geographic origin and used material from various counties across four states to ensure that we accounted for different populations and intraspecies variability. In addition to “target” specimens that we used in the primary morphological analysis, we examined an additional 20 females and 20 males of each species (“secondary morphological exemplars;” Suppl. material 1: table 1) to confirm the characters given in descriptions and obtain replicate measurements of body length for each species. We provide raw morphological data (Suppl. material 1: table 4), and a summary of characters, states, and corresponding diagnostic utility (Suppl. material 1: table 5). We report morphological characters in taxon treatments as character-state pairs matching the findings of the morphological “test”.

We performed morphological observations and measurements of mounted specimens with an Olympus SZX16 stereo microscope (Olympus Life Science, Tokyo, Japan) fitted with an optical micrometer. We measured antennae at a resolution of 0.005 (1/200) millimeters using 10 \times magnification in combination with the 2 \times objective. Other measurements were taken at an appropriate magnification using the 1 \times objective. We used a gooseneck illuminator fitted with mylar strips to diffuse light, which

was especially helpful for discerning minute patterns involving sculpture and other aspects of the cuticular surface.

Terms relating to gall phenotypes follow Deans et al. (2023).

Imaging and drawings

We took images of point-mounted adult wasps using a Macroscopic Solutions ‘micro-kit’ (Tolland, CT) imaging system. Additional images were captured from mounted wasps using Olympus SZX16 microscope (Olympus Life Science, Tokyo, Japan). We stacked images using Zerene Stacker LLC (Richland, WA), edited them using Adobe Photoshop (Adobe Inc.), and prepared plates using Adobe Illustrator (Adobe Inc.).

Nomenclature of host plants

For taxonomy of *Silphium* host plants we follow Brock and Weakley (2020). While taxonomy of *Silphium* is widely considered unsettled, the four species considered here (*Silphium integrifolium*, *S. laciniatum*, *S. perfoliatum*, and *S. terebinthinaceum*) are robust in the geographic area that we considered (M. Brock, in litt.).

Distribution of gall wasps and host plants

We generated maps showing confirmed and potential distribution of each gall wasp species using MapChart (figures are licensed via CC BY-SA 4.0). We defined potential distribution of each gall wasp species as the known native range of its host plant; distribution of *Silphium* species follows Kartesz (2015).

Molecular phylogenetics

We sequenced and analyzed the *cytochrome c oxidase subunit I* (COI) gene to test the species concepts suggested by our morphological analysis and host plant data. We sequenced the COI gene of three individuals of each species of the *Antistrophus rufus* complex. We also sequenced single specimens of three additional *Antistrophus* species: *A. silphii* Gillette, 1891 reared from a terminal stem gall on *Silphium integrifolium* Michx. (Fig. 1D), *A. laciniatus* Gillette, 1891 reared from a flower gall on *Silphium laciniatum* (Fig. 1F), and *A. microseris* (McCracken & Egbert, 1922) reared from a stem gall on *Microseris douglasii* (DC.) Sch.Bip. We also included a single sequence of *Isococcus leuzeae* Nieves-Aldrey, 2003 from GenBank ([DQ012643](#)) to serve as outgroups to the *A. rufus* complex.

We performed DNA extraction using an E.Z.N.A. Microelute Genomic DNA Kit (Omega Bioteck Inc., Norcross, Georgia, USA) following kit protocols and eluting in 30 µL of buffer (15 µL eluted in two steps). We extracted DNA from wasps either entirely destructively, by grinding the entire body, or minimally destructively, by incubating the entire insect. We amplified the COI gene using primer pairs LEPR

and LEPF (Hebert et al. 2004) or LCO1490 and HCO2198 (Folmer et al. 1994), following Hebert et al.'s PCR conditions for LEP primers and following those of Pang et al. (2020) for Folmer primers. Sequencing of PCR products was performed using The Huck Institute's Genomics Core Facility (The Pennsylvania State University, University Park, PA). We edited and aligned sequence data using Geneious (Biomatters Ltd., Auckland, New Zealand, <http://www.geneious.com/>) using the Geneious alignment function. Aligned sequence ends were trimmed to exclude primer sequences. We calculated genetic distances in Geneious using the Tamura-Nei distance model and 1,000 bootstrap replications. We determined the number of variable and parsimony informative sites in our alignment using AMAS v1.0 (Borowiec, 2016).

We estimated phylogenetic relationships using MrBayes v3.2.7a (Ronquist et al. 2012) using the following parameters: ngen=15000000; samplefreq=1000; nchains=4; nrungs=3. We used two partitions for the first+second and third codon positions, respectively, and selected nucleotide substitution models for the two partitions using PartitionFinder 2 (Lanfear et al. 2017) using a greedy search (Lanfear et al. 2012) and specifying for models available in MrBayes; PartitionFinder 2 recommended GTR+G for the first+second positions and HKY+G for the third position. We specified *I. leuzeae* as the outgroup and used a burnin value of 3750000 corresponding to 25% of samples. We visualized our consensus tree using FigTree v1.4.4 (<https://tree.bio.ed.ac.uk/software/figtree/>).

All new sequences generated for this study were deposited in GenBank, accessions [PP739172–PP739189](#). We provide collection data for sequenced specimens (Suppl. material 1: table 6) and DNA barcode divergence data (Suppl. material 1: table 7). We deposited DNA vouchers and additional material from gall samples and/or rearing events at PSUC.

Identification of additional museum material

After developing diagnostic characters for each studied species of *Antistrophus*, we identified additional material of the *A. rufus* species complex from collections we referenced, with the goal of checking previous determinations of identified specimens and identifying undetermined specimens for the first time.

Results

Morphological evaluation

Of the 50 characters that we assessed, 13 had utility as diagnostic characters for at least one species of the *rufus* complex. Two of the diagnostic characters, both of which concerned dimensions of the first two flagellomeres, were used previously by Tooker et al. (2004) in their treatment of the complex; our observations of those characters were generally consistent with those of Tooker et al. (2004).

While the specimens that we reared from *S. laciniatum*, *S. perfoliatum*, and *S. terebinthinaceum* all matched existing species concepts, the wasps associated with *S. integrifolium* appeared to compose an independent species, and our morphological observations suggest significant differences between these individuals and those of the described members of the *rufus* complex. Based on these differences, and the results of our molecular phylogenetic analysis, we describe below the species associated with *S. integrifolium* as *Antistrophus laurenae* Nastasi, sp. nov.

Molecular phylogenetics

Our trimmed alignment was 623 bp in length and contained 216 variable sites and 187 parsimony informative sites. Our Bayesian phylogenetic analysis of the 19 sequences (Fig. 2) provided clear evidence of distinct species matching those found by our morphological data, as each of the species had very short branch lengths within species (maximum COI divergence of 1.4% in *A. meganae*) and very long branch lengths between them. COI species divergences between the closest species pair (*A. meganae* and *A. minor*) averaged 10.6% (Suppl. material 1: table 7), well beyond the often contested 2% ‘suggestion’ for distinguishing species. Each species was supported by a posterior

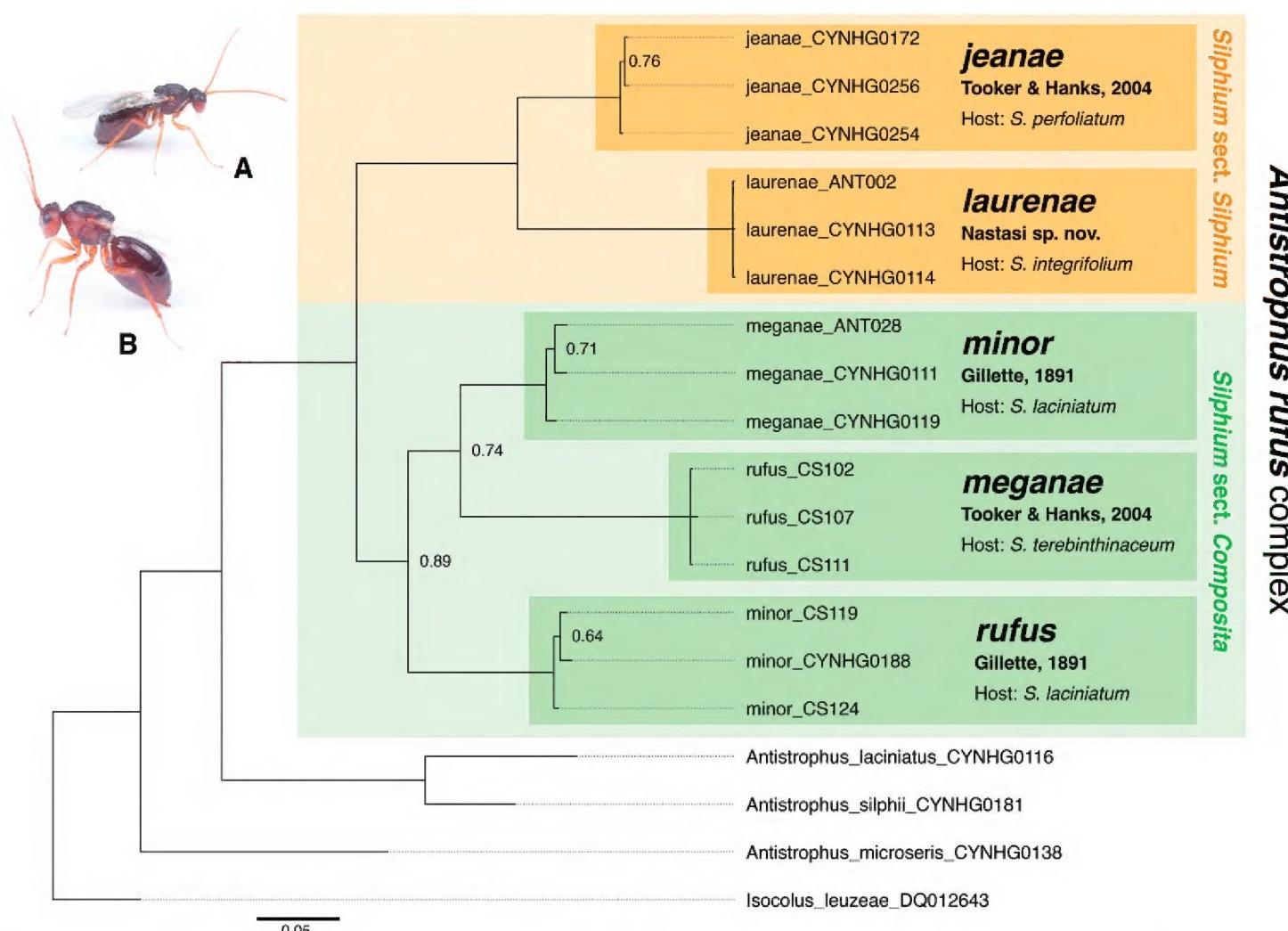


Figure 2. Bayesian phylogenetic tree of DNA barcodes for the *Antistrophus rufus* complex. Support values shown only for nodes with posterior probability < 1 . Host plant clade associations are indicated by colored boxes (*Silphium* sect. *Silphium* in orange and *S. sect. Composita* in green) **A** adult female *Antistrophus jeanae* Tooker & Hanks, 2004 **B** adult female *Antistrophus rufus* Gillette, 1891.

probability of 1, and *A. laurenae* and *A. jeanae* were strongly supported as sister species, but the remainder of the between-species relationships are less robust. Each recognized species has a distinct host plant except for *A. rufus* and *A. minor*, which are both associated with *Silphium laciniatum* but were not supported as sister species. Our analysis also retrieved the *Antistrophus rufus* complex as monophyletic within the sampled species of *Antistrophus* with a posterior probability of 1.

Taxonomy of the *Antistrophus rufus* species complex

Antistrophus rufus species complex

Diagnosis. Overall, the *A. rufus* complex is best diagnosed by the following combination of characters: head and mesosoma mostly reddish-brown in color (Figs 3A–D); facial radiating striae complete (Fig. 3A); compound eye longer than malar space in anterior view (Fig. 3A); F2 much longer than F1 (e.g., Fig. 4B); mesopleuron sculpture reticulate with fine intermediate striae (Fig. 3B); notauli incomplete, indistinct in anterior third of mesoscutum (Fig. 3C); female fore wing without apparent marginal setae (Fig. 3D); galls inconspicuous, induced in stems of *Silphium* species (e.g., Fig. 4F). Comparative diagnostic characters for the *Antistrophus rufus* species complex relative to other *Antistrophus* are presented in Fig. 3 and Table 2 below.

Table 2. Diagnostic characters of the *Antistrophus rufus* complex. Morphological data for species outside the *A. rufus* complex are from ongoing revisionary work on Aulacideini (Nastasi et al., unpublished data). Biological data are from Nastasi and Deans (2021). * = Gall and host unconfirmed for *A. bicolor* Gillette, 1891; see Nastasi and Deans (2021). † = Coloration of fresh material unknown; based on description by Beutenmüller (1908).

<i>Antistrophus</i> species	Head and mesosoma coloration	Facial radiating striae	Malar space: eye	F2:F1 length	Mesopleuron sculpture	Notauli	Fore wing marginal setae (♀)	Gall morphotype and host plant
<i>rufus</i> complex (<i>jeanae</i> , <i>laurenae</i> , <i>meganae</i> , <i>minor</i> , & <i>rufus</i>)	Mostly reddish-brown	Complete, reaching eye (Fig. 3A)	Eye longer (Fig. 3A)	F2 much longer (e.g., Fig. 4B)	Reticulate with fine intermediate striae (Fig. 3B)	Incomplete, indistinct in anterior third (Fig. 3C)	Absent (Fig. 3D)	Inconspicuous, in stems of <i>Silphium</i> (e.g., Fig. 4F)
<i>bicolor</i> , <i>laciniatus</i> , & <i>silphii</i>	Entirely black	Complete	Eye longer	F2 subequal to F1	Reticulate with fine intermediate striae	Complete, distinct across mesoscutum length	Absent	Conspicuous, on stems or flowers of <i>Silphium</i> *
<i>chrysanthami</i>	Black to reddish- brown†	Absent	Eye longer	F2 much longer	Strongly striate with interspaces reticulate	Incomplete, distinct only in posterior third	Absent	Conspicuous, apparently on <i>Chrysanthamus</i>
<i>microseris</i>	Entirely black	Complete	Eye longer	F2 much longer	Reticulate with fine intermediate striae	Incomplete, distinct only in posterior third	Present	Conspicuous, on stems of <i>Microseris</i>
<i>pisum</i>	Mostly reddish-brown	Incomplete, reaching halfway to eye	Malar space longer	F2 much longer	Entirely reticulate	Incomplete, distinct only in posterior third	Absent	Conspicuous, on stems of <i>Lygodesmia</i>



Figure 3. Diagnostic characters of the *Antistrophus rufus* complex **A** head, anterior view, *A. meganae* female (PSUC_FEM_247320); frs = facial radiating striae **B** mesopleuron, lateral view, *A. laurenae* female (PSUC_FEM_248174) **C** mesoscutum, dorsal view, *A. rufus* female (PSUC_FEM_253129); not = notauli; arrow indicates end of distinct portion **D** fore wing, *A. minor* female (PSUC_FEM_253176).

Remarks. The *Antistrophus rufus* complex includes all species of *Antistrophus* known to induce inconspicuous galls in stems of *Silphium* species. While other species of *Antistrophus* induce galls on *Silphium*, they induce perceptible galls on the apical stems or in the flowerheads (Gillette 1891; Nastasi and Deans 2021) and are generally distinct morphologically (Table 2). Other species of *Antistrophus* induce galls on different host genera. A complete revision of *Antistrophus* and other North American Aulacideini is being undertaken by the authors (Nastasi et al., unpublished data).

Antistrophus jeanae Tooker & Hanks, 2004

Fig. 4

Antistrophus jeanae Tooker & Hanks, 2004 in Tooker et al. 2004: 132. ♀, ♂ (type locality: Buckley Railroad Prairie, Iroquois Co., Illinois, USA).

Material examined. **Holotype** (deposited at INHS). USA • ♀; Illinois, Iroquois County, Buckley Railroad Prairie; 40°34.88'N, 88°02.70'W; J. Tooker leg.; reared from stem of *Silphium perfoliatum* in June 2000; INHS Insect Collection 25845.



Figure 4. *Antistrophus jeanae* Tooker & Hanks, 2004 **A** female lateral habitus (PSUC_FEM_248169) **B** female proximal antennomeres (PSUC_FEM_248395) **C** female dorsal head (PSUC_FEM_247240) **D** female dorsal mesosoma (PSUC_FEM_247240) **E** female mesoscutum (PSUC_FEM_247240); not = notaulus **F** female dorsal scutellum (PSUC_FEM_247240); scf = mesoscutellar fovea **G** male lateral habitus (PSUC_FEM_248416) **H** gallss and larvae of *A. jeanae* in a stem of *Silphium perfoliatum* L.

Paratypes (14 ♀ and 8 ♂). Deposited at INHS: USA • 5 ♀ and 2 ♂; same data as for holotype; INHS Insect Collection 52846–52852 • 4 ♀ and 3 ♂; Illinois, Ford County, Paxton Railroad Prairie; 40°26.17'N, 88°06.36'W; J. Tooker leg.; reared from stems of *Silphium perfoliatum*, emerging in June 2000; INHS Insect Collection 52853–52859.

Deposited at USNM: USA • 2 ♀ and 1 ♂; same data as for holotype; USNMENT 01790168–01790170 • 3 ♀ and 2 ♂; Illinois, Ford County, Paxton Railroad Prairie; 40°26.17'N, 88°06.36'W; J. Tooker leg.; reared from stems of *Silphium perfoliatum*, emerging in June 2000; USNMENT 00961127; 01790165–01790167; 01790171.

Other material (10 ♀ and 17 ♂). Deposited at PSUC: USA • 3 ♀ and 1 ♂; Illinois, Champaign County, Mahomet, Buffalo Trace Prairie; 40.208, -88.411; galled plant material collected 11 Nov 2020; JF Tooker and AR Deans leg.; reared from stems of *Silphium perfoliatum*, emerging in May or June 2021; PSUC_FEM_248395; 248398; 248413; 248423 • 4 ♀ and 3 ♂; Indiana, Parke County; 39.661, -87.371; galled plant material collected 12 Nov 2020; JF Tooker and AR Deans leg.; reared from stems of *Silphium perfoliatum*, emerging in May or June 2021; PSUC_FEM_248169–248170; 248396–248397; 248401–248403 • 2 ♀ and 3 ♂; Iowa, Winneshiek County, Plymouth Rock Prairie; 43.437, -92.005; galled plant material collected 19 Nov 2020; MJ Hatfield leg.; reared from stems of *Silphium perfoliatum*, emerging in May 2021; PSUC_FEM_248406; 248416; 248418; 248420; 248422.

Deposited at WIRC: 4 ♂; Wisconsin, Dane County, Anthony Branch F.A.; 42.896072, -89.340018; galled plant material collected 4 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium perfoliatum*, emerging in Apr 2012; WIRC 00171031–00171032; 00171035–00171036 • 2 ♂; Wisconsin, Dane County, Badger Pr. Park - North; galled plant material collected 3 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium perfoliatum*, emerging in Apr 2012; WIRC 00171041; 00171043 • 1 ♂; Wisconsin, Dane County, E-way at Mooreland; galled plant material collected 4 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium perfoliatum*, emerging in Apr 2012; WIRC 00171047 • 1 ♀; Wisconsin, Iowa County, Noll Valley; galled plant material collected 4 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium perfoliatum*, emerging in Apr 2012; WIRC 00170518 • 3 ♂; Wisconsin, Iowa County, Pr. Grove Rd at stream; galled plant material collected 5 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium perfoliatum*, emerging in Apr 2012; WIRC 00171027–00171029.

Diagnosis. *A. jeanae* is most similar to *A. laurenae* sp. nov. but is best distinguished by the dimensions of F2 in females, which in *A. jeanae* is 3.8× as long as wide (Fig. 4B) but 3.3× as long as wide in *A. laurenae* (Fig. 5B). The sculpture of the mesoscutellar disc (Fig. 4F) is also useful; the mesoscutellar disc is more or less rugose-reticulate throughout in *A. jeanae* but only has rugose-reticulate sculpture toward the outer margins in *A. laurenae*.

Description. Female (Fig. 4A)–**Body length:** 1.9–3.2 mm ($\bar{x} = 2.7$ mm; $n = 25$; holotype = 2.6 mm). **Color:** Antenna color: red brown throughout, at most slightly darker distally than proximally. Head color: vertex and occiput dark red brown, mandi-

bles red brown basally to darker red brown apically, rest of head red brown throughout. Mesosoma color: pronotum and propodeum red brown laterally to dark red brown medially, mesoscutum dark red brown, scutellum dark red brown, and mesopleuron dark red brown dorsally and ventrally but red brown medially. Wing membrane color: hyaline throughout. Wing vein color: light brown. Leg color: red brown throughout, except for apical tarsomere which is conspicuously darker. Metasoma color: red brown to dark red brown. **Antennae** (Fig. 4B): Antennomere count: 13. F1 length: 2.4× as long as wide. F2 length: 3.8× as long as wide. F2:F1 length ratio: 1.5. Placodeal sensilla on F2: absent; sensilla present only on F3 and following antennomeres. **Head** (Fig. 4C): Upper frons sculpture: reticulate. Gena posterolateral projection in anterior view: distinctly projecting past compound eyes. Facial radiating striae: distinct and complete, reaching compound eyes. Supraclypeal area sculpture: reticulate. Supraclypeal area projection: slightly projecting. OOL vs POL: OOL distinctly longer. OOL vs LOL: OOL greater than twice LOL. POL vs LOL: POL greater than twice LOL. LOL vs DLO: LOL longer. Vertex sculpture: reticulate throughout. Clypeus sculpture: reticulate. **Mesosoma** (Figs 4D–F): Pronotum pilosity: densely pilose along anterior margin and with only sparse setae elsewhere. Pronotum excluding pronotal plate sculpture: reticulate. Pronotal plate sculpture: reticulate. Mesopleuron excluding speculum sculpture: reticulate with fine intermediate striae. Speculum sculpture: reticulate. Mesopleuron pilosity: ventral margin and mesopleural triangle densely pilose and bare elsewhere. Mesoscutum pilosity: sparsely pilose. Mesoscutum sculpture: reticulate. Apparent length of anterior parallel lines: reaching one third across mesoscutum. Morphology of anterior parallel lines: narrow, distinct throughout perceptible length. Apparent length of parapsidal grooves: reaching halfway across mesoscutum. Morphology of parapsidal grooves: narrow, distinct throughout perceptible length. Morphology of median mesoscutal impression: apparent as a shallow impression extending across most of mesoscutum. Notauli completeness: incomplete, distinct posteriorly to indistinct in anterior third. Morphology of notauli: appearing as wide indentations with sloping edges throughout distinct portions. Metapleural sulcus: meeting posterior mesopleuron at about one third of its height. Lateral propodeal carinae: distinct and subparallel. Metapleuron sculpture: reticulate. Mesoscutellar foveae distinction: distinct, relatively deep anteriorly to shallower and inconspicuously delimited posteriorly. Mesoscutellar foveae sculpture: reticulate. Mesoscutellar disc sculpture: usually rugose-reticulate throughout; primarily reticulate, but usually with distinct rugose sculpture apparent throughout most of mesoscutellar disc. Mesoscutellar foveae length: reaching about one third across mesoscutellar disc. Mesoscutellar foveae shape: subquadrate, about as wide as long, separated by a narrow linear carina. Mesoscutellar disc shape: subcircular, about as wide as long. **Wings:** Marginal cell length: 3.2× as long as wide. Fore wing distal fringe of marginal setae: absent. **Metasoma:** Punctuation of metasomal terga: T3 punctate only in posterior third and with T4 and following punctate throughout.

Male (Fig. 4G)—Same as female except for the following: **Body length:** 1.8–2.6 mm ($\bar{x} = 2.1$ mm; $n = 25$). **Antennae:** Antennomere count: 14. F1 length: 2.4× as long as wide. F2 length: 2.9× as long as wide. F2:F1 length ratio: 1.4. Placodeal sensilla

on F2: present. **Wings:** Fore wing distal fringe of marginal setae: present. **Metasoma:** Metasoma size: conspicuously smaller than in female.

Biology. *A. jeanae* induces inconspicuous, externally imperceptible galls in stems of *Silphium perfoliatum* (Fig. 4H) (Tooker et al. 2004; Nastasi and Deans 2021).

Distribution. Tooker et al. (2004) reported this species only from several prairie sites in Illinois (USA). Nastasi and Deans (2021) did not report additional localities. However, specimens we examined revealed records from three additional state records: Indiana, Iowa, and Wisconsin. A verifiable iNaturalist observation (<https://www.inaturalist.org/observations/114414672>) also records this species from Ohio; we have since examined adult gall wasps reared from the plant material from the same site, which confirmed their suspected identity (in litt.). Lastly, a specimen identified during this study (USNMENT 01822302; see complete specimen data in Suppl. material 1: table 1) confirms the occurrence of this species in Missouri (Columbia, Boone County) (see discussion). Known and potential distribution are summarized in Fig. 9.

Antistrophus laurenae Nastasi, sp. nov.

<https://zoobank.org/3EA96644-BC93-4B03-908C-C62BEAABDCDF>

Fig. 5

Material examined. **Holotype** (deposited at PSUC). USA • ♀; Illinois, Ford and Iroquois Counties, Paxton Railroad Prairie; 40.359, -88.106; galled plant material collected 11 November 2020; JF Tooker and AR Deans leg.; reared from stems of *Silphium integrifolium*, emerging in May or June 2021; PSUC_FEM_248174.

Paratypes (24 ♀ and 25 ♂). Deposited at PSUC: USA • 2 ♂; same data as for holotype; PSUC_FEM_248338–248339 • 1 ♀ and 4 ♂; Illinois, Iroquois County, Loda Cemetery Prairie; 40.528, -88.074; galled plant material collected 10 November 2020; JF Tooker and AR Deans leg.; reared from stems of *Silphium integrifolium*, emerging in May or June 2021; PSUC_FEM_248173 • 5 ♀ and 4 ♂; Illinois, McLean County, Weston Cemetery Prairie; 40.725, -88.606; galled plant material collected 11 November 2020; JF Tooker and AR Deans leg.; reared from stems of *Silphium integrifolium*, emerging in May or June 2021; PSUC_FEM_248178; 248325–248327; 248329–248330; 248333; 248336 • 2 ♀ and 2 ♂; Iowa, Story County, Grant, intersection of I-35 and E 13th St. prairie restoration; galled plant material collected 28 November 2020; MJ Hatfield leg.; reared from stems of *Silphium integrifolium*, emerging in June 2021; PSUC_FEM_248176; 248331; 248334; 248337.

Deposited at USNM: USA • 1 ♀; Illinois, McLean County, Weston Cemetery Prairie; 40.725, -88.606; galled plant material collected 11 November 2020; JF Tooker and AR Deans leg.; reared from stems of *Silphium integrifolium*, emerging in May or June 2021; PSUC_FEM_248332 • 1 ♀ and 2 ♂; same data as for holotype; PSUC_FEM_248175; 248335; 248340.

Deposited at WIRC: 2 ♀ and 1 ♂; Iowa, Delaware County, Dyersville West; 42.48564, -91.15425; galled plant material collected 2 May 2009; DNR Study SSGB

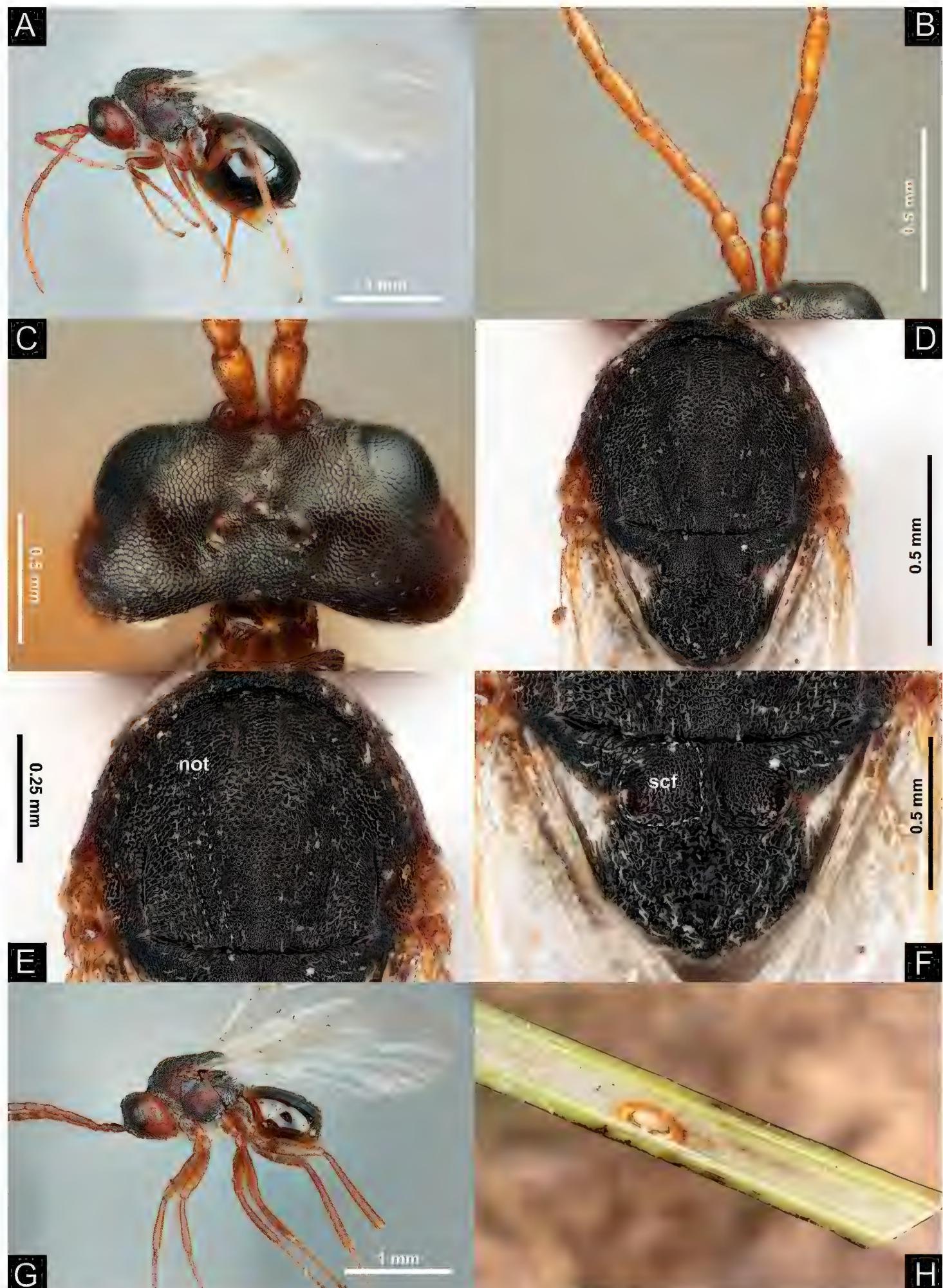


Figure 5. *Antistrophus laurenae* Nastasi, sp. nov. **A** paratype female lateral habitus (PSUC_FEM_248173) **B** holotype female proximal antennomeres (PSUC_FEM_248174) **C** holotype female dorsal head (PSUC_FEM_248174) **D** holotype female dorsal mesosoma **E** holotype female dorsal mesoscutum; not = notaulus **G** holotype female dorsal scutellum; scf = mesoscutellar fovea. Paratype male lateral habitus (PSUC_FEM_248334) **H** galls and larva of *A. laurenae* in a stem of *Silphium integrifolium* Michx.

leg.; reared from stems of *Silphium integrifolium*, emerging in May 2009; WIRC 00171299–00171301 • 2 ♀ and 1 ♂; Iowa, Delaware County, Dyersville West Prairie; 42.47330, -91.138372; galled plant material collected 2 May 2009; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in May 2009; WIRC 00171267–00171268; 00171283 • 1 ♀ and 2 ♂; Iowa, Marshall County, Twinkle Hill Restoration; 42.064396, -92.854943; galled plant material collected 2 May 2009; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in May 2009; WIRC 00171284; 00171306–00171307 • 1 ♀; Wisconsin, Columbia County, Goose Pond Jill's Pr.; galled plant material collected 3 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in Apr 2012; WIRC 00171314 • 1 ♂; Wisconsin, Dane County, Anthony Branch F.A.; 42.896072, -89.340018; galled plant material collected 4 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in Apr 2012; WIRC 00171322 • 2 ♀ and 2 ♂; Wisconsin, Dane County, Badger Pr. Park - North; 42.896072, -89.340018; galled plant material collected 3 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in Apr 2012; WIRC 00170523; 00170837; 00171315; 00171331 • 1 ♀; Wisconsin, Dane County, Black Earth Prairie; 43.140146, -89.773011; galled plant material collected 5 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in Apr 2012; WIRC 00170838 • 1 ♀ and 1 ♂; Wisconsin, Dane County, E-way at Mooreland; 43.030588, -89.349114; galled plant material collected 4 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in Apr 2012; WIRC 00170524; 00171329 • 1 ♀ and 1 ♂; Wisconsin, Dane County, Festge Roadside; 43.118267, -89.683665; galled plant material collected 18 Apr 2009; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in May 2009; WIRC 00171227; 00171289 • 1 ♂; Wisconsin, Dane County, Garfoot Road; galled plant material collected 5 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in Apr 2012; WIRC 00171337 • 2 ♀ and 2 ♂; Wisconsin, Dane County, Gov Nelson 1997; 43.137321, -89.437257; galled plant material collected 3 Apr 2012; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in Apr 2012; WIRC 00170839; 00171303; 00171330; 00171341 • 2 ♀ and 1 ♂; Wisconsin, Dane County, TNC Waubesa Wetland; galled plant material collected 22 Nov 2008; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in May 2009; WIRC 00171271; 00171274; 00171286 • 1 ♂; Wisconsin, Iowa County, TNC/Lease; 42.97357, -89.88900; galled plant material collected 17 Apr 2009; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in May 2009; WIRC 00171287 • 1 ♂; Wisconsin, Iowa County, Underwood Prairie; galled plant material collected 9 May 2009; DNR Study SSGB leg.; reared from stems of *Silphium integrifolium*, emerging in May 2009; WIRC 00171298.

Diagnosis. See diagnosis to *Antistrophus jeanae* Tooker & Hanks, 2004.

Description. Female (Fig. 5A)—**Body length:** 1.8–2.6 mm ($\bar{x} = 2.2$ mm; $n = 25$; holotype = 2.6 mm). **Color:** Antenna color: red brown throughout, at most slightly darker distally than proximally. Head color: vertex and occiput dark red brown, mandi-

bles red brown basally to darker red brown apically, rest of head red brown throughout. Mesosoma color: pronotum and propodeum red brown laterally to dark red brown medially, mesoscutum dark red brown, scutellum dark red brown, and mesopleuron dark red brown dorsally and ventrally but red brown medially. Wing membrane color: hyaline throughout. Wing vein color: light brown. Leg color: red brown throughout, except for apical tarsomere which is conspicuously darker. Metasoma color: red brown to dark red brown. **Antennae** (Fig. 5B): Antennomere count: 13. F1 length: 2.2× as long as wide. F2 length: 3.3× as long as wide. F2:F1 length ratio: 1.4. Placodeal sensilla on F2: absent; sensilla present only on F3 and following antennomeres. **Head** (Fig. 5C): Upper frons sculpture: reticulate. Gena posterolateral projection in anterior view: distinctly projecting past compound eyes. Facial radiating striae: distinct and complete, reaching compound eyes. Supraclypeal area sculpture: reticulate. Supraclypeal area projection: slightly projecting. OOL vs POL: OOL distinctly longer. OOL vs LOL: OOL greater than twice LOL. POL vs LOL: POL greater than twice LOL. LOL vs DLO: LOL longer. Vertex sculpture: reticulate throughout. Clypeus sculpture: reticulate. **Mesosoma** (Figs 5D–F): Pronotum pilosity: densely pilose along anterior margin and with only sparse setae elsewhere. Pronotum excluding pronotal plate sculpture: reticulate. Pronotal plate sculpture: reticulate. Mesopleuron excluding speculum sculpture: reticulate with fine intermediate striae. Speculum sculpture: reticulate. Mesopleuron pilosity: ventral margin and mesopleural triangle densely pilose and bare elsewhere. Mesoscutum pilosity: sparsely pilose. Mesoscutum sculpture: reticulate. Apparent length of anterior parallel lines: reaching one third across mesoscutum. Morphology of anterior parallel lines: narrow, distinct throughout perceptible length. Apparent length of parapsidal grooves: reaching halfway across mesoscutum. Morphology of parapsidal grooves: narrow, distinct throughout perceptible length. Morphology of median mesoscutal impression: apparent as a shallow impression extending across most of mesoscutum. Notauli completeness: incomplete, distinct posteriorly to indistinct in anterior third. Morphology of notauli: appearing as wide indentations with sloping edges throughout distinct portions. Metapleural sulcus: meeting posterior mesopleuron at about one third of its height. Lateral propodeal carinae: distinct and subparallel. Metapleuron sculpture: reticulate. Mesoscutellar foveae distinction: distinct, relatively deep anteriorly to shallower and inconspicuously delimited posteriorly. Mesoscutellar foveae sculpture: reticulate. Mesoscutellar disc sculpture: rugose-reticulate, primarily reticulate, but with distinct rugose-reticulate sculpture seemingly restricted to outer margins. Mesoscutellar foveae length: long, reaching about one third across mesoscutellar disc. Mesoscutellar foveae shape: subquadrate, about as wide as long, separated by a narrow linear carina. Mesoscutellar disc shape: subcircular, about as wide as long. **Wings:** Marginal cell length: 3.1× as long as wide. Fore wing distal fringe of marginal setae: absent. **Metasoma:** Punctuation of metasomal terga: T3 punctate only in posterior third and with T4 and following punctate throughout.

Male (Fig. 5G)—Same as female except for the following: **Body length:** 1.3–2.1 mm ($\bar{x} = 1.8$; $n = 25$). **Antennae:** Antennomere count: 14. F1 length: 2.3× as long as wide. F2 length: 3.2× as long as wide. F2:F1 length ratio: 1.5. Placodeal sensilla

on F2: present. **Wings:** Fore wing distal fringe of marginal setae: present. **Metasoma:** Metasoma size: conspicuously smaller than in female.

Etymology. Named for Lauren Ahlert, a biology teacher at Wayne Valley High School in Wayne, New Jersey, USA, who has served as a tremendous source of inspiration and passion for the author of this species.

Biology. *Antistrophus laurenae* induces inconspicuous, externally imperceptible galls in stems of *Silphium integrifolium* (Fig. 5H).

Distribution. Material examined in this study reveals that this species occurs in Illinois, Iowa, and Wisconsin (USA; see Suppl. material 1: table 1). A verified iNaturalist record (<https://www.inaturalist.org/observations/136446787>) also suggests that this species occurs in Mississippi. Known and potential distribution are summarized in Fig. 9.

Antistrophus meganae Tooker & Hanks, 2004

Fig. 6

Antistrophus meganae Tooker & Hanks, 2004 in Tooker et al. 2004: 132. ♀, ♂ (type locality: East St. Joseph Railroad Prairie, Iroquois Co., Illinois, USA).

Material examined. **Holotype** (deposited at INHS). USA • ♀; Illinois, Champaign County, St. Joseph, roadside prairie; J. Tooker leg.; reared from stem of *Silphium terebinthinaceum*, emerging in June 2000; INHS Insect Collection 52830.

Paratypes (14 ♀ and 8 ♂). Deposited at INHS: USA • 8 ♀ and 4 ♂; same data as for holotype; INHS Insect Collection 52831–52837 • 4 ♀ and 3 ♂; Illinois, Ford County, Paxton, Paxton Railroad Prairie; J. Tooker leg.; reared from stem of *Silphium terebinthinaceum*, emerging in June 2000; INHS Insect Collection 52838–52844.

Deposited at USNM: USA • 2 ♀ and 1 ♂; Illinois, Ford County, Paxton, Paxton Railroad Prairie; J. Tooker leg.; reared from stem of *Silphium terebinthinaceum*, emerging in June 2000; USNMENT [01790188–01790190](#).

Other material (10 ♀ and 17 ♂). Deposited at PSUC: USA • 1 ♀; Illinois, Champaign County, St. Joseph, roadside prairie; JF Tooker and AR Deans leg.; 40.113, -88.064; galled plant material collected 10 Nov 2020; reared from stem of *Silphium terebinthinaceum*, emerging in June 2021; PSUC_FEM_248429 • 1 ♀ and 1 ♂; Illinois, McLean County, Chenoa, Weston Cemetery Prairie; 40.725, -88.606; galled plant material collected 11 Nov 2020; reared from stem of *Silphium terebinthinaceum*, emerging in June 2021; PSUC_FEM_248165–248166.

Deposited at WIRC: USA • 2 ♀; Wisconsin, Columbia County, Mass Rd.; galled plant material collected Fall 2008; reared from stem of *Silphium terebinthinaceum*, emerging in June 2009; WIRC 00171156; 00171166 • 2 ♀ and 2 ♂; Wisconsin, Dane County, Co A and Oak Ridge (Anthony Branch F.A.); 42.892454, -89.320011; galled plant material collected 15 Apr 2009; reared from stem of *Silphium terebinthinaceum*, emerging in June 2009; WIRC 00170514; 00171140; 00171154–00171155 • 1 ♂;

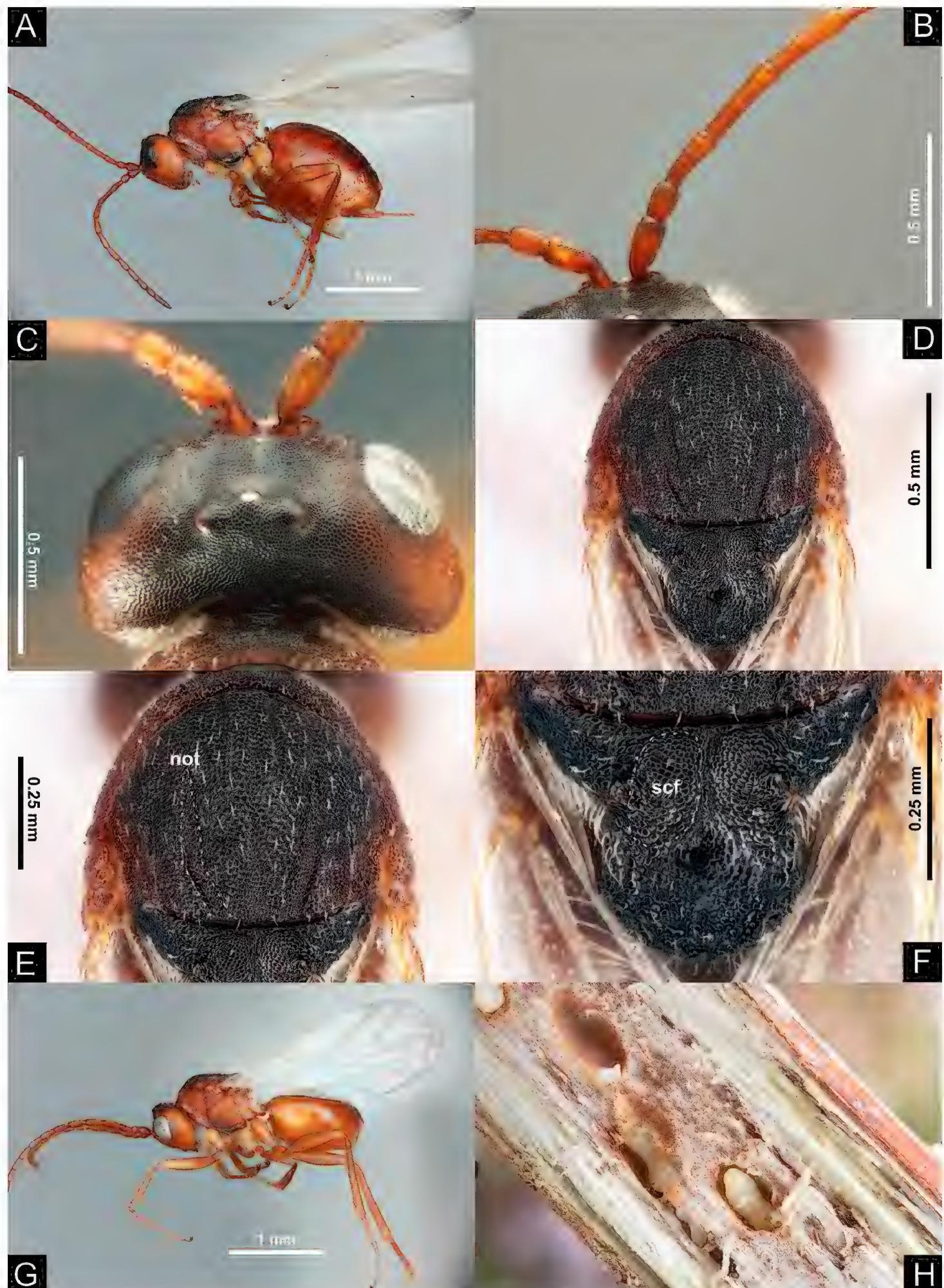


Figure 6. *Antistrophus meganae* Tooker & Hanks, 2004 **A** female lateral habitus (PSUC_FEM_248165) **B** female proximal antennomeres (PSUC_FEM_247325) **C** female dorsal head (PSUC_FEM_247325) **D** female dorsal mesosoma (PSUC_FEM_247230) **E** female dorsal mesoscutum (PSUC_FEM_247230); not = notaulus **F** female dorsal scutellum (PSUC_FEM_247230); scf = mesoscutellar fovea **G** male lateral habitus (PSUC_FEM_248498) **H** gallss and larvae of *A. meganae* in a stem of *Silphium terebinthinaceum* Jacq.

Wisconsin, Dane County, Kelly Road and RR, roadside; galled plant material collected Fall 2008; reared from stem of *Silphium terebinthinaceum*, emerging in July 2009; WIRC 00170513 • 1 ♂; Wisconsin, Dane County, Malone Road; galled plant material collected 17 Apr 2009; reared from stem of *Silphium terebinthinaceum*, emerging in June 2009; WIRC 00171112 • 1 ♂; Wisconsin, Dane County, Noll Valley; galled plant material collected 5 Apr 2012; reared from stem of *Silphium terebinthinaceum*, emerging in June 2012; WIRC 00171114 • 1 ♂; Wisconsin, Dane County, Prairie Ridge City Park; galled plant material collected 3 Apr 2012; reared from stem of *Silphium terebinthinaceum*, emerging in May 2012; WIRC 00171124 • 1 ♀ and 1 ♂; Wisconsin, Dane County, Sugar Ridge Savanna; galled plant material collected 20 Mar 2012; reared from stem of *Silphium terebinthinaceum*, emerging in May 2012; WIRC 00171122; 00171134 • 1 ♂; Wisconsin, Dane County, TNC Waubesa Wetland; galled plant material collected 22 Nov 2008; reared from stem of *Silphium terebinthinaceum*, emerging in May 2009; WIRC 00170515 • 1 ♂; Wisconsin, Jefferson County, Bluejoint Prairie; 43.163398, -88.938815; galled plant material collected 16 Apr 2009; reared from stem of *Silphium terebinthinaceum*, emerging in July 2009; WIRC 00170517 • 1 ♂; Wisconsin, Jefferson County, Bluejoint Prairie; 43.163398, -88.938815; galled plant material collected 16 Apr 2009; reared from stem of *Silphium terebinthinaceum*, emerging in May 2009; WIRC 00170512 • 1 ♀ and 2 ♂; Wisconsin, Jefferson County, Cold Spring Prairie; 42.872556, -88.770194; galled plant material collected 15 Apr 2009; reared from stem of *Silphium terebinthinaceum*, emerging in June 2009; WIRC 00171109; 00171137; 00171141 • 3 ♂; Wisconsin, Walworth County, Skoponong Prairie; 42.829155, -88.620586; galled plant material collected 4 Oct 2011; reared from stem of *Silphium terebinthinaceum*, emerging in May or June 2012; WIRC 00170749; 00171113; 00171120 • 1 ♀; Wisconsin, Walworth County, Young Pr. East Annex; 42.839419, -88.63002; galled plant material collected 16 Apr 2009; reared from stem of *Silphium terebinthinaceum*, emerging in June 2009; WIRC 00170750 • 1 ♀ and 2 ♂; Wisconsin, Winnebago County, Oshkosh-Larsen Trl mid (B); 44.13912, -88.624272; galled plant material collected 10 Oct 2011; reared from stem of *Silphium terebinthinaceum*, emerging in June 2012; WIRC 00171115–00171116; 00171118.

Diagnosis. *A. meganae* is the only species of the *rufus* complex in which the mesoscutellar foveae (Fig. 6F) are long and ovate, reaching nearly halfway across the mesoscutellar disc. *A. meganae* also differs from most other members of the *rufus* complex by the POL (Fig. 6C), which is longer than the OOL in *A. meganae* and *A. minor* but shorter than the OOL in the other species.

Description. Female (Fig. 6A)–**Body length:** 1.8–3.2 mm ($\bar{x} = 2.5$ mm; $n = 25$; holotype = 2.3 mm). **Color:** Antenna color: red brown throughout, at most slightly darker distally than proximally. Head color: vertex and occiput dark red brown, mandibles red brown basally to darker red brown apically, rest of head red brown throughout. Mesosoma color: pronotum and propodeum red brown laterally to dark red brown medially, mesoscutum dark red brown with distinct posterolateral red brown spots, scutellum dark red brown, and mesopleuron dark red brown dorsally

and ventrally but red brown medially. Wing membrane color: hyaline throughout. Wing vein color: light brown. Leg color: red brown throughout, except for apical tarsomere which is conspicuously darker. Metasoma color: red brown to dark red brown. **Antennae** (Fig. 6B): Antennomere count: 13. F1 length: 2.6× as long as wide. F2 length: 3.6× as long as wide. F2:F1 length ratio: 1.4. Placodeal sensilla on F2: absent; sensilla present only on F3 and following antennomeres. **Head** (Fig. 6C): Upper frons sculpture: reticulate. Gena posterolateral projection in anterior view: distinctly projecting past compound eyes. Facial radiating striae: distinct and complete, reaching compound eyes. Supraclypeal area sculpture: reticulate. Supraclypeal area projection: slightly projecting. OOL vs POL: POL distinctly longer. OOL vs LOL: OOL less than twice LOL. POL vs LOL: POL twice LOL. LOL vs DLO: LOL longer. Vertex sculpture: reticulate throughout. Clypeus sculpture: reticulate. **Mesosoma** (Figs 6D–F): Pronotum pilosity: densely pilose along anterior margin and with only sparse setae elsewhere. Pronotum excluding pronotal plate sculpture: reticulate. Pronotal plate sculpture: reticulate. Mesopleuron excluding speculum sculpture: reticulate with fine intermediate striae. Speculum sculpture: reticulate. Mesopleuron pilosity: ventral margin and mesopleural triangle densely pilose and bare elsewhere. Mesoscutum pilosity: sparsely pilose. Mesoscutum sculpture: reticulate. Apparent length of anterior parallel lines: reaching one third across mesoscutum. Morphology of anterior parallel lines: narrow, distinct throughout perceptible length. Apparent length of parapsidal grooves: reaching halfway across mesoscutum. Morphology of parapsidal grooves: narrow, distinct throughout perceptible length. Morphology of median mesoscutal impression: apparent as a shallow impression extending across most of mesoscutum. Notauli completeness: incomplete, distinct posteriorly to indistinct in anterior third. Morphology of notauli: appearing as wide indentations with sloping edges throughout distinct portions. Metapleural sulcus: meeting posterior mesopleuron at about one third of its height. Lateral propodeal carinae: distinct and subparallel. Metapleuron sculpture: reticulate. Mesoscutellar foveae distinction: distinct, relatively deep anteriorly to shallower and inconspicuously delimited posteriorly. Mesoscutellar foveae sculpture: reticulate. Mesoscutellar disc sculpture: rugose-reticulate, primarily reticulate, but with distinct rugose-reticulate sculpture seemingly restricted to outer margins. Mesoscutellar foveae length: long, reaching nearly halfway across mesoscutellar disc. Mesoscutellar foveae shape: ovate, slightly longer than wide, separated by a subtriangular carina slightly wider posteriorly than anteriorly. Mesoscutellar disc shape: subcircular, about as wide as long. **Wings**: Marginal cell length: 3.3× as long as wide. Fore wing distal fringe of marginal setae: absent. **Metasoma**: Punctuation of metasomal terga: T3 punctate only in posterior third and with T4 and following punctate throughout.

Male (Fig. 6G)—Same as female except for the following: **Body length:** 1.3–2.5 mm ($\bar{x} = 2.1$ mm; $n = 25$). **Antennae**: Antennomere count: 14. F1 length: 2.5× as long as wide. F2 length: 3.0× as long as wide. F2:F1 length ratio: 1.4 (as in female). Placodeal sensilla on F2: present. **Wings**: Fore wing distal fringe of marginal setae: present. **Metasoma**: Metasoma size: conspicuously smaller than in female.

Biology. *Antistrophus meganae* induces inconspicuous, externally imperceptible galls in stems of *Silphium terebinthinaceum* (Fig. 6H) (Tooker et al. 2004; Nastasi and Deans 2021).

Distribution. Tooker et al. (2004) reported this species only from several prairie sites in Illinois (USA). Nastasi and Deans (2021) did not report additional localities; however, the specimens we examined revealed novel records from Wisconsin (Suppl. material 1: table 1). We also sequenced DNA barcodes for individuals from Ohio (Suppl. material 1: table 6), and a series of specimens identified using the key represents a new state record from Michigan (see Discussion; Suppl. material 1: table 1). Known and potential distribution are summarized in Fig. 9.

Antistrophus minor Gillette, 1891

Fig. 7

Antistrophus minor Gillette, 1891: 195. ♀, ♂ (type locality: unknown location in Illinois, USA).

Aulax gillettei (Gillette, 1891): Kieffer 1902: 93.

Material examined. **Lectotype** (deposited at INHS; designated by Frison [1927]). USA • ♀; Illinois; reared from stem of *Silphium laciniatum*; record number 5500; INHS Insect Collection 212949.

Lectoallotype (deposited at INHS; designated by Frison [1927]). USA • ♂; same data as lectotype; INHS Insect Collection 212950.

Other material (24 ♀ and 24 ♂). Deposited at PSUC: USA • 3 ♀; Illinois, Champaign County, Gifford, Shortline Railroad Prairie; 40.305, -87.999; JF Tooker and AR Deans leg.; galled plant material collected 10–11 Nov 2020; reared from stem of *Silphium laciniatum*, emerging in May or June 2021; PSUC_FEM_248257; 253063; 253067 • 2 ♀; Illinois, Iroquois County, Loda, Loda Cemetery Prairie; 40.528, -88.074; JF Tooker and AR Deans leg.; galled plant material collected 10 Nov 2020; reared from stem of *Silphium laciniatum*, emerging in June 2021; PSUC_FEM_248273; 253060 • 2 ♀ and 1 ♂; Illinois, McLean County, Chenoa, Weston Cemetery Prairie; 40.725, -88.606; JF Tooker and AR Deans leg.; galled plant material collected 11 Nov 2020; reared from stem of *Silphium laciniatum*, emerging in May or June 2021; PSUC_FEM_248278; 248283; 248288; 248303 • 3 ♀ and 3 ♂; Illinois, McLean County, Chenoa, Weston Cemetery Prairie; 40.725, -88.606; LF Nastasi and AR Casadei leg.; galled plant material collected 16 Oct 2021; reared from stem of *Silphium laciniatum*, emerging in June 2022; PSUC_FEM_253166–253168; 253170; 253180; 253192 • 1 ♀ and 2 ♂; Iowa, Story County, Grant, Interstate 35 and E 13th St prairie restoration; MJ Hatfield leg.; galled plant material collected 30 Nov 2020; reared from stem of *Silphium laciniatum*, emerging in May or June 2021; PSUC_FEM_248265; 248291; 248295 • 1 ♂; Iowa, Winneshiek County, Decorah Community Prairie; MJ Hatfield leg.; galled plant material collected 18 Apr 2022; reared from

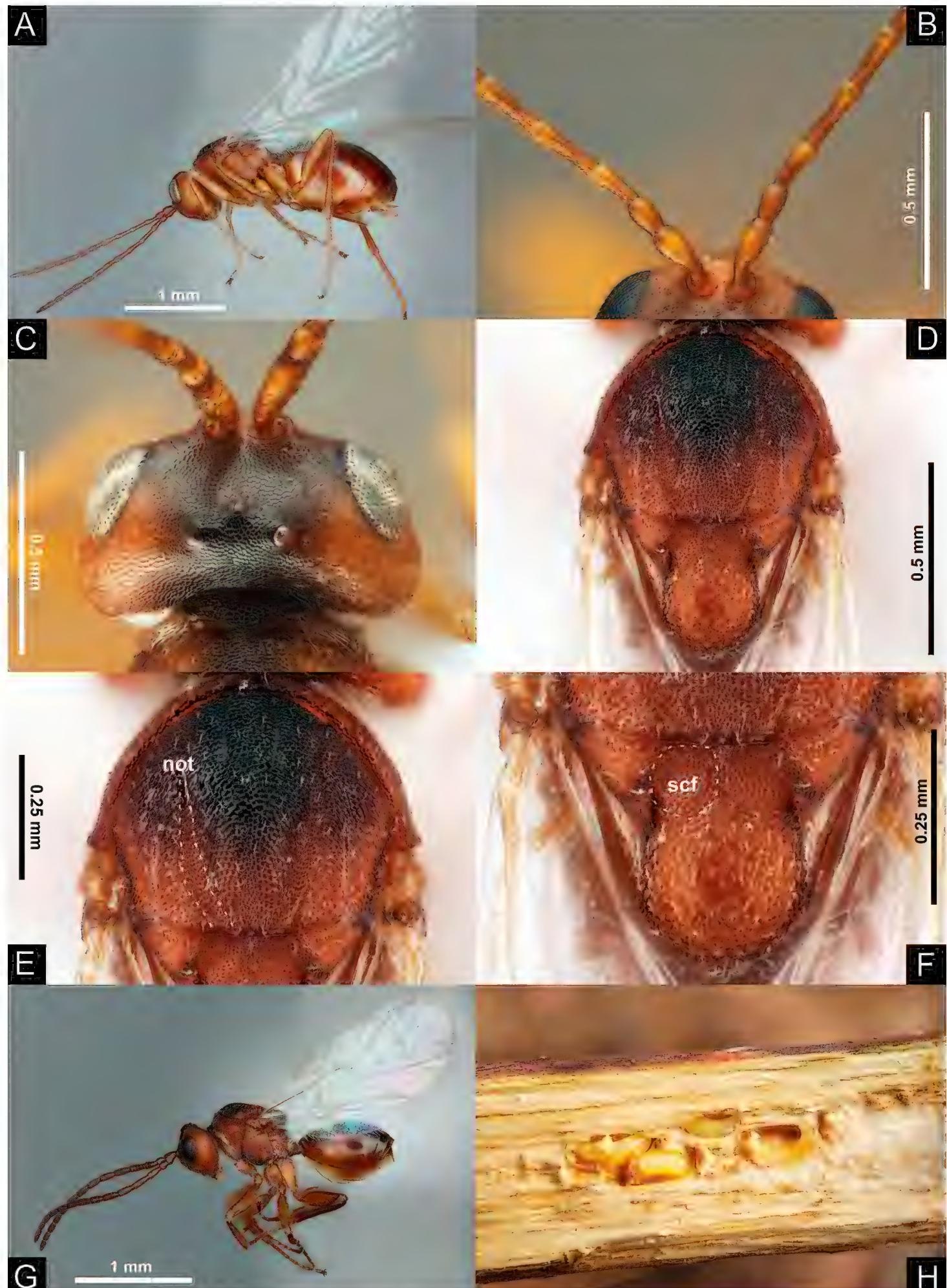


Figure 7. *Antistrophus minor* Gillette, 1891 **A** female lateral habitus (PSUC_FEM_248273) **B** female proximal antennomeres (PSUC_FEM_253176) **C** female dorsal head (PSUC_FEM_253091) **D** female dorsal mesosoma (PSUC_FEM_248257) **E** female dorsal mesoscutum (PSUC_FEM_248257); not = notaulus **F** female dorsal scutellum (PSUC_FEM_248257); scf = mesoscutellar fovea **G** male lateral habitus (PSUC_FEM_253191) **H** gallss and larvae of *A. minor* or *A. rufus* in a stem of *Silphium laciniatum* L.

stem of *Silphium laciniatum*, emerging in May or June 2022; PSUC_FEM_253229 • 3 ♀ and 2 ♂; Iowa, Winneshiek County, Plymouth Rock Prairie; 43.438, -92.006; MJ Hatfield leg.; galled plant material collected 17 Apr 2016; reared from stem of *Silphium laciniatum*, emerging in May 2016; PSUC_FEM_253085; 253088; 253089; 253090; 253094.

Deposited at USNM: USA • 1 ♀; Illinois, McLean County, Chenoa, Weston Cemetery Prairie; 40.725, -88.606; JF Tooker and AR Deans leg.; galled plant material collected 11 Nov 2020; reared from stem of *Silphium laciniatum*, emerging in May or June 2021; PSUC_FEM_248286 • 1 ♀; Illinois, McLean County, Chenoa, Weston Cemetery Prairie; 40.725, -88.606; JF Tooker and AR Deans leg.; galled plant material collected 11 Nov 2020; reared from stem of *Silphium laciniatum*, emerging in May or June 2021; PSUC_FEM_248303 • 3 ♂; Illinois, McLean County, Chenoa, Weston Cemetery Prairie; 40.725, -88.606; LF Nastasi and AR Casadei leg.; galled plant material collected 16 Oct 2021; reared from stem of *Silphium laciniatum*, emerging in June 2022; PSUC_FEM_253190; 253191; 253194 • 1 ♀; Illinois, McLean County, Chenoa, Weston Cemetery Prairie; 40.725, -88.606; LF Nastasi and AR Casadei leg.; galled plant material collected 16 Oct 2021; reared from stem of *Silphium laciniatum*, emerging in June 2022; PSUC_FEM_253165.

Deposited at WIRC: USA • 1 ♀; Iowa, Blackhawk County, Hudson Railroad Prairie; 42.436349, -92.427034; DNR Study SSGB leg.; galled plant material collected 2 May 2009; reared from stem of *Silphium laciniatum*, emerging in June 2009; WIRC 00170819 • 1 ♀; Iowa, Linn County, Walford 2; 41.883014, -91.825962; DNR Study SSGB leg.; galled plant material collected 3 May 2009; reared from stem of *Silphium laciniatum*, emerging in June 2009; WIRC 00170772 • 1 ♂; Wisconsin, Columbia County, Goose Pd. Sue Ames; DNR Study SSGB leg.; galled plant material collected Fall 2008; reared from stem of *Silphium laciniatum*, emerging in July 2009; WIRC 00170816 • 1 ♀; Wisconsin, Columbia County, Hopkins Restoration; DNR Study SSGB leg.; galled plant material collected Fall 2008; reared from stem of *Silphium laciniatum*, emerging in July 2009; WIRC 00170661 • 1 ♂; Wisconsin, Dane County, Badger Pr. Park North; DNR Study SSGB leg.; galled plant material collected 3 Apr 2012; reared from stem of *Silphium laciniatum*, emerging in May 2012; WIRC 00170757 • 1 ♂; Wisconsin, Dane County, E-way @ Mooreland; DNR Study SSGB leg.; galled plant material collected 4 Apr 2012; reared from stem of *Silphium laciniatum*, emerging in Apr 2012; WIRC 00170683 • 2 ♀; Wisconsin, Dane County, Smith Drumlin; 42.98988, -89.06097; DNR Study SSGB leg.; galled plant material collected 15 Apr 2009; reared from stem of *Silphium laciniatum*, emerging in May 2009; WIRC 00170786; 00170788 • 2 ♂; Wisconsin, Dane County, Waubesa Wetlands; WDNR Misc/SB Sauer leg.; galled plant material collected Apr 2007; reared from stem of *Silphium laciniatum*, emerging before 19 May 2007; WIRC 00170805; 00170807 • 2 ♂; Wisconsin, Grant County, Dewey Heights Prairie; 42.734647, -91.020306; DNR Study SSGB leg.; galled plant material collected 14 Oct 2011; reared from stem of *Silphium laciniatum*, emerging in May 2012; WIRC 00170664; 00171400 • 2 ♀; Wisconsin, Green County, Green Cemetery Prairie; DNR Study

SSGB leg.; galled plant material collected 4 Apr 2012; reared from stem of *Silphium laciniatum*, emerging in May 2012; WIRC 00170663; 00170758 • 1 ♂; Wisconsin, Green County, Muralt - South; 42.695505, -89.49367; DNR Study SSGB leg.; galled plant material collected 4 Apr 2012; reared from stem of *Silphium laciniatum*, emerging in May 2012; WIRC 00170754 • 1 ♂; Wisconsin, Iowa County, Hollandale Seed Orchard; DNR Study SSGB leg.; galled plant material collected 5 Apr 2012; reared from stem of *Silphium laciniatum*, emerging in May 2012; WIRC 00170756 • 1 ♂; Wisconsin, Iowa County, TNC/Lease; 42.97357, -89.88900; DNR Study SSGB leg.; galled plant material collected 17 Apr 2009; reared from stem of *Silphium laciniatum*, emerging in May 2009; WIRC 00170678 • 1 ♂; Wisconsin, Kenosha County, Bain Station; 42.556352, -87.893763; DNR Study SSGB leg.; galled plant material collected 30 Sep 2011; reared from stem of *Silphium laciniatum*, emerging in May 2012; WIRC 00170778 • 1 ♀; Wisconsin, Waukesha County, Scuppernong Prairie State Natural Area; 42.89947, -88.50180; DNR Study SSGB leg.; galled plant material collected 15 Apr 2009; reared from stem of *Silphium laciniatum*, emerging in June 2009; WIRC 00170637.

Diagnosis. *A. minor* is the only described species of *Antistrophus* in which the mesoscutellar disc is strongly ovate and distinctly wider than long (Fig. 7F). Additionally, *A. minor* is easily separable from *A. rufus* (which also induces inconspicuous galls in stems of the *S. laciniatum*) and *A. meganae* by the shape and length of the mesoscutellar foveae; in *A. minor*, the mesoscutellar foveae are subquadrate and reach about one third across the mesoscutellar disc (Fig. 7F) but are shorter and subrectangular in *A. rufus* (Fig. 8F) and longer and ovate in *A. meganae* (Fig. 6F). *A. minor* and *A. meganae* are also the only species of the *rufus* complex in which the POL is longer than the OOL. See additional comments in the diagnosis to *A. rufus*.

Description. Female (Fig. 7A)–**Body length:** 1.4–2.4 mm ($\bar{x} = 2.0$ mm; $n = 25$; lectotype = 2.3). **Color:** Antenna color: red brown throughout, at most slightly darker distally than proximally. Head color: vertex and occiput dark red brown, mandibles red brown basally to darker red brown apically, rest of head red brown throughout. Color: pronotum and propodeum mostly red brown, with some dark red brown coloration medially, mesoscutum dark red brown with at least distinct red brown spots posterolaterally, scutellum red brown to dark red brown, and mesopleuron dark red brown to red brown. Wing membrane color: hyaline throughout. Wing vein color: light brown. Leg color: red brown throughout, except for apical tarsomere which is conspicuously darker. Metasoma color: red brown to dark red brown. **Antennae** (Fig. 7B): Antennomere count: 13. F1 length: 2.6× as long as wide. F2 length: 3.6× as long as wide. F2:F1 length ratio: 1.4. Placodeal sensilla on F2: absent; sensilla present only on F3 and following antennomeres. **Head** (Fig. 7C): Upper frons sculpture: reticulate. Gena posterolateral projection in anterior view: distinctly projecting past compound eyes. Facial radiating striae: distinct and complete, reaching compound eyes. Supraclypeal area sculpture: reticulate. Supraclypeal area projection: slightly projecting. OOL vs POL: POL distinctly longer. OOL vs LOL: OOL twice LOL. POL vs LOL: POL greater than twice LOL. LOL vs DLO: LOL longer. Vertex sculpture:

reticulate throughout. Clypeus sculpture: reticulate. **Mesosoma** (Figs 7D–F): Pronotum pilosity: densely pilose along anterior margin and with only sparse setae elsewhere. Pronotum excluding pronotal plate sculpture: reticulate. Pronotal plate sculpture: reticulate. Mesopleuron excluding speculum sculpture: reticulate with fine intermediate striae. Speculum sculpture: reticulate. Mesopleuron pilosity: ventral margin and mesopleural triangle densely pilose and bare elsewhere. Mesoscutum pilosity: sparsely pilose. Mesoscutum sculpture: reticulate. Apparent length of anterior parallel lines: reaching one third across mesoscutum. Morphology of anterior parallel lines: narrow, distinct throughout perceptible length. Apparent length of parapsidal grooves: reaching halfway across mesoscutum. Morphology of parapsidal grooves: narrow, distinct throughout perceptible length. Morphology of median mesoscutal impression: apparent as a shallow impression extending across most of mesoscutum. Notauli completeness: incomplete, distinct posteriorly to indistinct in anterior third. Morphology of notauli: appearing as wide indentations with sloping edges throughout distinct portions. Metapleural sulcus: meeting posterior mesopleuron at about one third of its height. Lateral propodeal carinae: distinct and subparallel. Metapleuron sculpture: reticulate. Mesoscutellar foveae distinction: distinct, relatively deep anteriorly to shallower and inconspicuously delimited posteriorly. Mesoscutellar foveae sculpture: reticulate. Mesoscutellar disc sculpture: rugose-reticulate, primarily reticulate, but with distinct rugose-reticulate sculpture seemingly restricted to outer margins. Mesoscutellar foveae length: long, reaching about one third across mesoscutellar disc. Mesoscutellar foveae shape: subovate, slightly longer than wide, separated by a narrow linear carina. Mesoscutellar disc shape: ovate, distinctly longer than wide. **Wings:** Marginal cell length: $3.1 \times$ as long as wide. Fore wing distal fringe of marginal setae: absent. **Metasoma:** Punctuation of metasomal terga: T3 punctate only in posterior third and with T4 and following punctate throughout.

Male (Fig. 7G)—Same as female except for the following: **Body length:** 1.1–2.0 mm ($\bar{x} = 1.6$; $n = 25$). **Antennae:** Antennomere count: 14. F1 length: $2.3 \times$ as long as wide. F2 length: $2.9 \times$ as long as wide. F2:F1 length ratio: 1.3. Placodeal sensilla on F2: present. **Wings:** Fore wing distal fringe of marginal setae: present. **Metasoma:** Metasoma size: conspicuously smaller than in female.

Biology. *Antistrophus minor* induces inconspicuous, externally imperceptible galls in stems of *Silphium laciniatum* (Fig. 7H) (Tooker et al. 2004; Nastasi and Deans 2021). Weld (1926) reported *A. minor* from *Silphium terebinthinaceum*, but Weld's specimens from this host plant truly represent *Antistrophus meganae*.

Distribution. *Antistrophus minor* was described from adults reared alongside *A. rufus* in Illinois. We examined specimens providing new state records from Iowa and Wisconsin. Known and potential distribution are summarized in Fig. 9. It appears that *A. minor* is likely sympatric with *A. rufus* throughout the distribution of *S. laciniatum*, although further rearing of *Silphium* species will be needed to better understand the role of geography in the distribution of *Antistrophus*, especially regarding these two species.

Remarks. See remarks under *Antistrophus rufus* for notes on generic nomenclature and distinction of *A. minor* from *A. rufus*.

***Antistrophus rufus* Gillette, 1891**

Fig. 8

Antistrophus rufus Gillette, 1891: 195. ♀, ♂ (type locality: unknown location in Illinois, USA).

Aulax rufa (Gillette, 1891): Kieffer 1902: 93.

Material examined. **Lectotype** (deposited at INHS; designated by Frison [1927]). USA • ♀; Illinois; reared from stem of *Silphium laciniatum*; record number 5500; INHS Insect Collection 52812.

Lectoallotype (deposited at INHS; designated by Frison [1927]). USA • ♂; same data as lectotype; INHS Insect Collection 52813.

Paralectotypes. Deposited at AMNH: USA • 1 ♀; same data as lectotype; AMNHIZC 00393875.

Deposited at INHS: USA • 3 ♀ and 1 ♂; same data as lectotype; INHS Insect Collection 294742–294745.

Deposited at USNM: USA • 4 ♀ and 2 ♂; same data as lectotype; USNMENT 00961146; 01822098–01822102.

Other material (16 ♀ and 21 ♂). Deposited at INHS: • 5 ♀ and 1 ♂; Illinois, Iroquois County, Buckley Railroad Prairie; J. Tooker leg.; reared from stem of *Silphium laciniatum*, emerging in June 2000; INHS Insect Collection 52815–52818; 52820–52821 • 1 ♀ and 1 ♂; Illinois, McLean County, Chenoa, Weston Cemetery Prairie; J. Tooker leg.; reared from stem of *Silphium laciniatum*, emerging in June 2000; INHS Insect Collection 18238–18239.

Deposited at PSUC: USA • 3 ♀ and 4 ♂; Illinois, Champaign County, Gifford, Shortline Railroad Prairie; 40.305, -87.999; JF Tooker and AR Deans leg.; galled plant material collected 10 Nov 2020; reared from stem of *Silphium laciniatum*, emerging in May or June 2021; PSUC_FEM_248301; 248307; 248310; 248315–248317 • 1 ♀ and 1 ♂; Illinois, McLean County, Chenoa, Weston Cemetery Prairie; 40.725, -88.606; JF Tooker and AR Deans leg.; galled plant material collected 11 Nov 2020; reared from stem of *Silphium laciniatum*, emerging in May or June 2021; PSUC_FEM_248293; 248296 • 1 ♀ and 7 ♂; Iowa, Story County, Grant, Interstate 35 and E 13th St. Prairie Restoration; MJ Hatfield leg.; galled plant material collected 30 Nov 2020; reared from stem of *Silphium laciniatum*, emerging in May or June 2021; PSUC_FEM_248163; 248308; 248311–248313; 248318–248320.

Deposited at USNM: USA • 1 ♀ and 2 ♂; Illinois, Ford County, Gifford, Ludlow Railroad Prairie; J. Tooker leg.; reared from stem of *Silphium laciniatum*, emerging in June 2000; USNMENT 01822106; 01822109–01822110 • 2 ♀ and 1 ♂; Illinois, Iroquois County, Buckley Railroad Prairie; J. Tooker leg.; reared from stem of *Silphium laciniatum*, emerging in June 2000; USNMENT 01822104; 01822107–01822108 • 7 ♀ and 2 ♂; Illinois, Vermillion County, Fithian Railroad Prairie; J. Tooker leg.; reared from stem of *Silphium laciniatum*, emerging in June 2000; INHS Insect Collection 52822–52828; UNSMENT 01822103; 01822105 • 1 ♂; Illinois, Evanston; reared; UNSMENT 01822112.

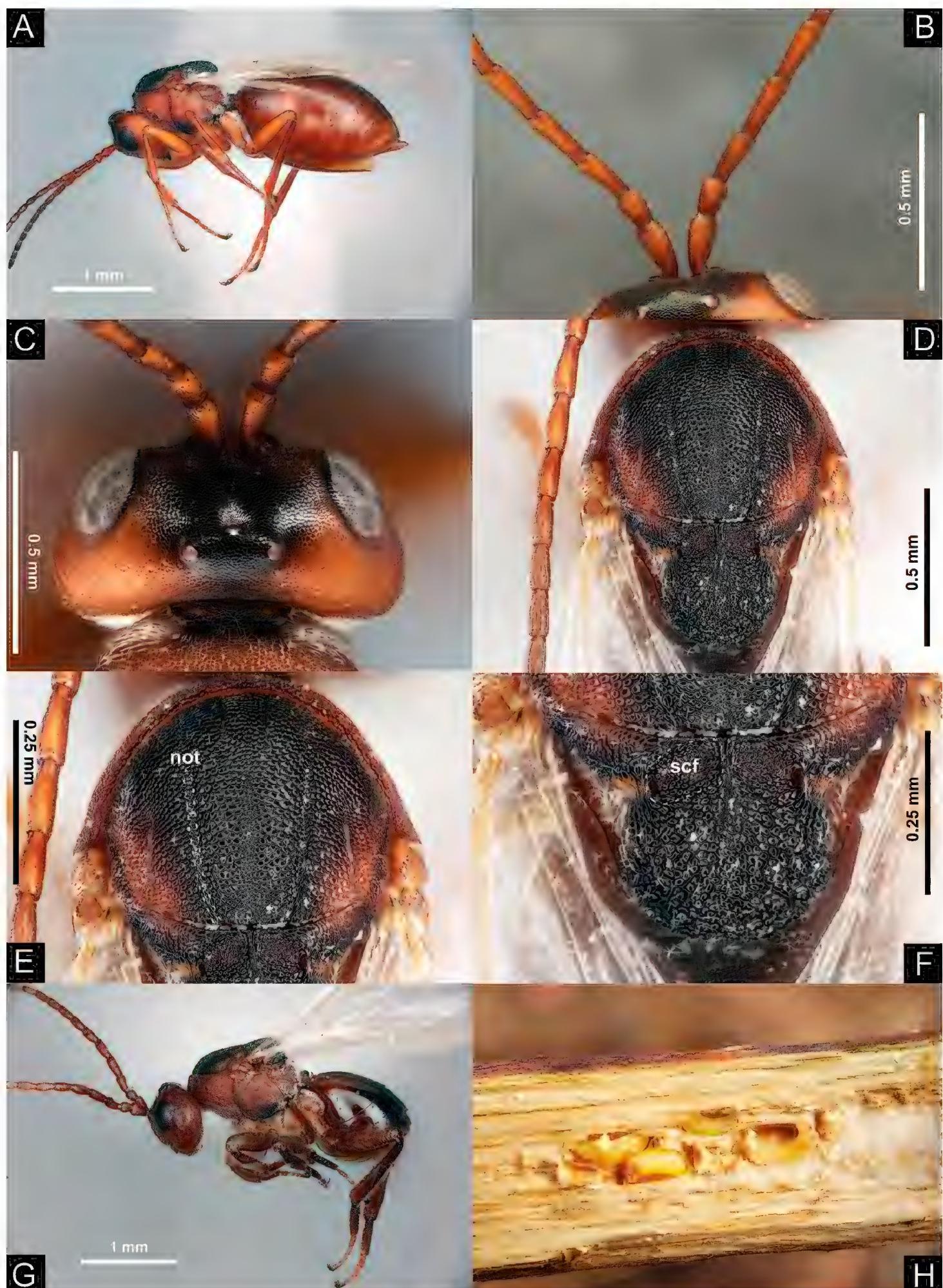
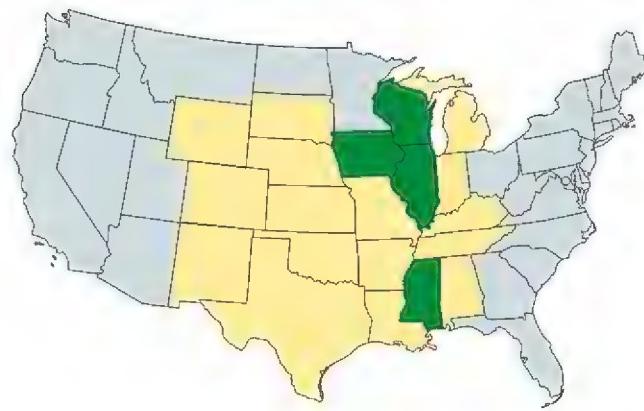
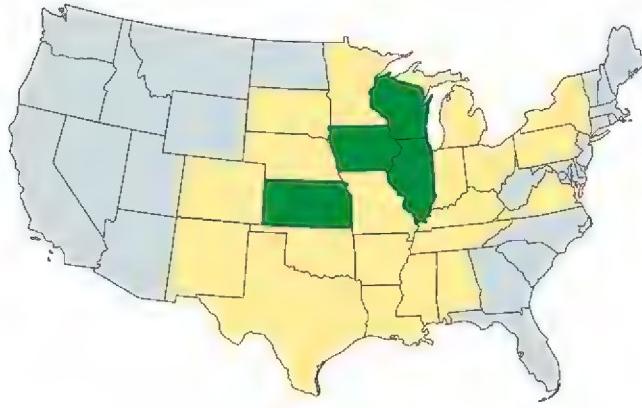


Figure 8. *Antistrophus rufus* Gillette, 1891 **A** female lateral habitus (PSUC_FEM_248316) **B** female proximal antennomeres (PSUC_FEM_246062) **C** female dorsal head (PSUC_FEM_246062) **D** female dorsal mesosoma (PSUC_FEM_253129) **E** female dorsal mesoscutum (PSUC_FEM_253129); not = notaulus **F** female dorsal scutellum (PSUC_FEM_253129); scf = mesoscutellar fovea **G** male lateral habitus (PSUC_FEM_248308) **H** gallss and larvae of *A. minor* or *A. rufus* in a stem of *Silphium laciniatum* L.

jeanae Tooker & Hanks, 2004*laurenae* Nastasi sp. nov.*meganae* Tooker & Hanks, 2004*minor* Gillette, 1891*rufus* Gillette, 1891

■ Confirmed gall wasp record
■ Known host plant distribution

Figure 9. Known and potential distributions for species of the *Antistrophus rufus* complex. Host plant distribution follows Kartesz (2015). States in green reflect established distribution, while states in yellow reflect potential distribution based on records of the host plant.

Deposited at WIRC: USA • 1 ♀; Wisconsin, Columbia County, Goose Pd. Bicentennial; DNR Study SSGB leg.; galled plant material collected Fall 2008; reared from stem of *Silphium laciniatum*, emerging in July 2009; WIRC 00170506 • 1 ♀; Wisconsin, Dane County, Thousands Rock Pt. Pr.; 42.99064, -89.8303; DNR Study SSGB leg.; galled plant material collected 17 Apr 2009; reared from stem of *Silphium laciniatum*, emerging in May 2009; WIRC 00170687 • 1 ♂; Wisconsin, Waukesha County, Scuppernong Prairie State Natural Area; 42.89947, -88.5018; DNR Study SSGB leg.; galled plant material collected 15 Apr 2009; reared from stem of *Silphium laciniatum*, emerging in Apr 2009; WIRC 00170643.

Diagnosis. *A. rufus* is easily recognized amongst members of the *rufus* complex by the shape and dimensions of the mesoscutellar foveae (Fig. 8F), which are short and

subrectangular in *A. rufus* but longer and subquadrate or ovate in the other species. The narrow, well-defined notauli (Fig. 8E) are also characteristic, as the other species all have wider and less-defined notauli. The smallest *A. rufus* specimens may exhibit slightly wider notauli, but the short, rectangular scutellar foveae are always apparent and readily distinguish this species from others. *A. rufus* also has the shortest F2 relative to its width in females (2.8× as long as wide; Fig. 8B)).

A. rufus are commonly reared alongside *A. minor* from inconspicuous, externally imperceptible galls in stems of *Silphium laciniatum* L.; these species are separated by the characters given above and those in the diagnosis of *A. minor*.

Description. Female (Fig. 8A)—**Body length:** 2.3–3.6 mm ($\bar{x} = 2.9$ mm; $n = 25$; lectotype = 2.8 mm). **Color:** Antenna color: red brown throughout, at most slightly darker distally than proximally. Head color: vertex and occiput dark red brown, mandibles red brown basally to darker red brown apically, rest of head red brown throughout. Mesosoma color: pronotum and propodeum red brown laterally to dark red brown medially, mesoscutum dark red brown with distinct posterolateral red brown spots, scutellum dark red brown, and mesopleuron dark red brown dorsally and ventrally but red brown medially. Wing membrane color: hyaline throughout. Wing vein color: light brown. Leg color: red brown throughout, except for apical tarsomere which is conspicuously darker. Metasoma color: red brown to dark red brown. **Antennae** (Fig. 8B): Antennomere count: 13. F1 length: 2.4× as long as wide. F2 length: 2.8× as long as wide. F2:F1 length ratio: 1.2. Placodeal sensilla on F2: absent; sensilla present only on F3 and following antennomeres. **Head** (Fig. 8C): Upper frons sculpture: reticulate. Gena posterolateral projection in anterior view: distinctly projecting past compound eyes. Facial radiating striae: distinct and complete, reaching compound eyes. Supraclypeal area sculpture: reticulate. Supraclypeal area projection: slightly projecting. OOL vs POL: OOL distinctly longer. OOL vs LOL: OOL greater than twice LOL. POL vs LOL: POL twice LOL. LOL vs DLO: LOL longer. Vertex sculpture: reticulate throughout. Clypeus sculpture: reticulate. **Mesosoma** (Figs 8D–F): Pronotum pilosity: densely pilose along anterior margin and with only sparse setae elsewhere. Pronotum excluding pronotal plate sculpture: reticulate. Pronotal plate sculpture: reticulate. Mesopleuron excluding speculum sculpture: reticulate with fine intermediate striae. Speculum sculpture: reticulate. Mesopleuron pilosity: ventral margin and mesopleural triangle densely pilose and bare elsewhere. Mesoscutum pilosity: sparsely pilose. Mesoscutum sculpture: reticulate. Apparent length of anterior parallel lines: reaching one third across mesoscutum. Morphology of anterior parallel lines: narrow, distinct throughout perceptible length. Apparent length of parapsidal grooves: reaching halfway across mesoscutum. Morphology of parapsidal grooves: narrow, distinct throughout perceptible length. Morphology of median mesoscutal impression: apparent as a shallow impression extending across most of mesoscutum. Notauli completeness: incomplete, distinct posteriorly to indistinct in anterior third. Morphology of notauli: appearing as narrow, well-delimited indentations throughout distinct portions. Metapleural sulcus: meeting posterior mesopleuron at about one third of its height. Lateral propodeal carinae: distinct and subparallel. Metapleuron sculpture: reticulate. Mesoscutellar foveae distinction: distinct, relatively deep anteriorly to shallower and inconspicuously delimited posteriorly. Mesoscutellar foveae sculpture: reticulate. Mesoscutellar disc sculpture: rugose-

reticulate, primarily reticulate, but with distinct rugose-reticulate sculpture seemingly restricted to outer margins. Mesoscutellar foveae length: short, occupying only anterior quarter of mesoscutellar disc. Mesoscutellar foveae shape: subrectangular, distinctly wider than long, and separated by a broad, elevated linear carina. Mesoscutellar disc shape: subcircular, about as wide as long. **Wings:** Marginal cell length: 2.7× as long as wide. Fore wing distal fringe of marginal setae: absent. **Metasoma:** Punctuation of metasomal terga: T3 punctate only in posterior third and with T4 and following punctate throughout.

Male (Fig. 8G)—Same as female except for the following: **Body length:** 1.9–3.5 mm ($\bar{x} = 2.5$ mm; $n = 25$). **Antennae:** Antennomere count: 14. F1 length: 2.1× as long as wide. F2 length: 2.5× as long as wide. F2:F1 length ratio: 1.3. Placodeal sensilla on F2: present. **Wings:** Fore wing distal fringe of marginal setae: present. **Metasoma:** Metasoma size: conspicuously smaller than in female.

Biology. *Antistrophus rufus* induces inconspicuous, externally imperceptible galls in stems of *Silphium laciniatum* (Fig. 8H) (Tooker et al. 2004; Nastasi and Deans 2021). Records associating *A. rufus* with other host plants most likely represent distinct species including the other species addressed in the present work.

Distribution. *Antistrophus rufus* was described from material collected in Illinois (USA) and has since been reported from Kansas (Tooker et al. 2004; Nastasi and Deans 2021). Additional material that we examined revealed additional records from Iowa and Wisconsin, and reiterated occurrence of this species in Kansas (Suppl. material 1: table 1). Known and potential distribution are summarized in Fig. 9.

Remarks. Kieffer (1902) synonymized *Antistrophus* with *Aylax* (=*Aulax*) Hartig, 1840. Some authors (e.g., Beutenmüller 1910) follow Kieffer's synonymy, but others appear to have rejected or otherwise omitted this change from many works treating the group (e.g., Weld 1951; Nieves-Aldrey 1994). This generic synonymy and the resulting species synonyms were accidentally omitted from Nastasi and Deans' (2021) catalogue, and we are unable to locate a nomenclatural act reinstating *Antistrophus* as a valid genus. The genus *Aylax* has now long been regarded to include only two species of gall wasps inducing galls on *Papaver* (Papaveraceae) (Nieves-Aldrey 1994; Ronquist et al. 2015). Additionally, the two genera are now placed in different tribes: *Aylax* is now placed in the tribe Aylacini, while *Antistrophus* is placed in the Aulacideini. We regard here *Antistrophus* as a valid genus independent of *Aylax*, although *Antistrophus* represents a heterogeneous assemblage as currently defined based on preliminary data from an ongoing revisionary study (Nastasi et al., unpublished data).

Lastly, two specimens labeled as paralectotypes in the USNM collection (USNMENT 01823001; 01823000) do not appear to be *Antistrophus rufus* and more closely resemble *A. minor*. They are both in relatively poor condition; at this time, their species identities cannot be substantiated due to damage to multiple body parts.

Key to the species of the *Antistrophus rufus* complex

Our assessment of diagnostic characters allowed for development of a key to the species of the *rufus* complex. A key to all species of *Antistrophus* is beyond the scope of this work but will be prepared as part of ongoing revisionary studies of North

American herb gall wasps (Nastasi et al., unpublished data). For best results using this key, we recommend using light diffusion in combination with high magnification (see Methods).

Before running a specimen through the below key, one should confirm that it is indeed an *Antistrophus* species using the keys in Nastasi et al. (2024) and/or Buffington et al. (2020) where appropriate, and then confirming that the specimen belongs to the *A. rufus* complex using the diagnostic criteria given in the diagnosis to the *A. rufus* complex and in Table 2.

- 1 Mesoscutellar disc (Fig. 7F) elongate and ovate, about 1.3× as long as wide. Galls in stems of *Silphium laciniatum*.....*A. minor*
- Mesoscutellar disc subcircular, about 1.1× as long as widest width (Figs 4F, 5F, 6F, 8F). Galls in stems of various *Silphium* including *S. laciniatum* 2
- 2 Mesoscutellar foveae (Fig. 8F) subrectangular, distinctly wider than long, and relatively short, only about one fourth as long as mesoscutellar disc. Notauli (Fig. 8E) usually apparent as narrower, well-defined lines throughout distinct portion. F2 of female about 2.8× as long as wide (Fig. 8B). Galls in stems of *Silphium laciniatum* *A. rufus*
- Mesoscutellar foveae subquadrate or ovate, at least as long as wide, and relatively longer, at least one third as long as mesoscutellar disc (Figs 4F, 5F, 6F). Notauli apparent as wider, sloping indentations throughout distinct portion (Figs 4E, 5E, 6E). F2 of female longer, at least 3.3× as long as wide. Galls in stems of other hosts 3
- 3 Mesoscutellar foveae (Fig. 6F) ovate, longer than wide, and reaching nearly halfway across mesoscutellar disc. POL longer than OOL (Fig. 6C). Galls in stems of *Silphium terebinthinaceum* *A. meganae*
- Mesoscutellar foveae subquadrate, occupying anterior third of mesoscutellar disc (Figs 5F, 6F). POL shorter than OOL (Figs 5C, 6C). Galls in stems of other hosts 4
- 4 F2 of female about 3.8× as long as wide (Fig. 4B). Mesoscutellar disc (Fig. 4F) usually with coarser sculpture, with sculpture mostly rugose-reticulate, especially medially. Galls in stems of *Silphium perfoliatum* *A. jeanae*
- F2 of female about 3.3× as long as wide (Fig. 5B). Mesoscutellar disc (Fig. 5F) mostly reticulate, with rugose sculpture restricted to outer margins. Galls in stems of *Silphium integrifolium* *A. laurenae*

Discussion

Our findings suggest that the species belonging to the *A. rufus* complex are not truly cryptic, and are readily identifiable with appropriate diagnostic characters. Other species complexes of herb gall wasps might benefit from a similar investigation of morphological species boundaries, especially species of *Aulacidea* Ashmead inducing galls on *Lactuca* L. or other *Antistrophus* inducing externally perceptible galls on terminal

stems and in flowers of *Silphium*. Such analyses would supplement the original descriptions and would help determine whether true cryptic species occur in Aulacideini.

While the taxonomic impediment impacts a tremendous number of species across all taxa, especially in groups such as “little brown beetles,” microlepidoptera, and many groups of parasitic wasps, our study of the *A. rufus* complex demonstrates that such issues can be disentangled with dedicated revisionary approaches. While no number of characters can ensure discovery of novel diagnostic characters, attempts to evaluate larger numbers of characters, as attempted here, may help decipher boundaries of cryptic species that would otherwise require molecular characters or additional methods. Examining 50 characters proved sufficient for the *rufus* complex, but for other taxa, it could be useful to apply a “brute force” approach involving a much greater number of candidate morphological characters. In such a scenario, different groups of characters could be tested iteratively until robust species diagnoses are achieved.

After developing the key to the *rufus* complex that we present above, we keyed and identified over 50 additional specimens belonging to the *rufus* complex from the INHS and USNM collections (Suppl. material 1: table 1), including both already determined and previously unidentified material. One series of specimens deposited at USNM represents a new state record of *A. meganae* from Michigan; these specimens’ labels suggesting they were collected off “elecampane” (presumably referring to *Inula helenium* L.), but this likely constitutes a misidentification of the proper host plant *Silphium terebinthinaceum* which is rather similar visually. We found a single specimen of *A. jeanae* amongst unsorted material in the USNM collection (USNMENT 01822302); this specimen was collected in Missouri and represents a new state record (see also remarks for *A. jeanae*).

Our examination indicates that *A. minor* is truly a species distinct from *A. rufus* despite previous suggestion that they may be synonymous. In various cynipid taxa, including Aulacideini, a single host plant species is galled by several distinct species of gall wasps (Nieves-Aldrey et al. 2004; Melika 2006; Nastasi and Deans 2021; Nastasi et al. 2024). However, few (if any) gall wasps share the same host plant while inhabiting the same plant organ and the same portion of gall phenospace (i.e., host plant organ combined with gall phenotype). While most of the *A. rufus* complex species each occupy a unique host plant species, the *Antistrophus rufus* complex appears to be somewhat unique among gall wasps, as two species both induce inconspicuous galls within the stems of the same host plant *S. laciniatum*. This suggests speciation mechanisms may be involved (e.g., geographic isolation or host switching) that may not follow the typically observed speciation in gall wasps, which normally is associated with shifts between host plants or gall morphotypes, at least in oak gall wasps (Cynipini) or their inquilines (Synergini *sensu lato*) (e.g., Ward et al. 2020, 2022, 2024).

Regarding evolution of host plant associations, the topology of our Bayesian analysis provides suggestion of cospeciation of *A. rufus* complex species with their host plants, as there was sorting of *A. rufus* complex species by host plant lineage (Fig. 2). Clevinger and Panero’s (2000) phylogenetic analysis of ETS and ITS markers suggested two major clades of *Silphium*; the *rufus* complex includes species associated with both

sections. Their sect. *Composita* includes species with deep taproots and prominent basal rosettes, including *S. laciniatum* and *S. terebinthinaceum*, while their sect. *Silphium* is composed mostly of species with more fibrous roots and without prominent basal rosettes, including *S. integrifolium* and *S. perfoliatum*. In our phylogenetic analysis, species galling sect. *Silphium* (*A. jeanae* and *A. laurenae*) formed a well-supported clade sister to the three species galling sect. *Composita* (*A. meganae*, *A. minor*, and *A. rufus*), although the latter clade was less strongly supported (posterior probability = 0.89).

More generally, our data show that each studied *Silphium* species has distinct species of *Antistrophus* occupying their stems. While the present study focused on the “big four” species of *Silphium* ubiquitous in prairies in the Midwestern U.S., a recent taxonomic treatment (Brock and Weakley 2020) suggests there are as many as 23 *Silphium* species; it is likely that more species of the *rufus* complex or other *Antistrophus* will be revealed in these other species. The deep divergences in DNA barcodes among these gall wasps suggest a long history of isolation and may indicate that additional species of the *A. rufus* complex await discovery and description. While interspecific divergences in our DNA barcode data are much higher than the supposed 2% “barcode gap” between animal species, other studies of diverse insect groups often find divergence in excess of 10% between congeneric species (Pentisaari et al. 2014; Lin et al. 2015; Song et al. 2018). Locating additional undescribed members of the *rufus* complex could aid in resolving relationships amongst the known species, and future research on host plant affinity and co-phylogeny between *Silphium* and their gall wasps will help inform understanding of these processes.

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Supplementary material I

Supplementary information

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Data type: (measurement/occurrence/multimedia/etc.)

Explanation note: **table 1.** Digitized complete specimen data for all material examined

table 2. Morphological characters examined **table 3.** Hymenoptera Anatomy Ontology URI table listing morphological terminology **table 4.** Raw morphological data **table 5.** Overview of diagnostic utility of examined characters **table 6.** Specimen data for sequenced material.

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